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SELF-INCOMPATIBILITY IN RYE

IV. FACTORS RELATED TO SELF-SEEDING

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(Received March 15th, 1958)

I. INTRODUCTION

RYE is generally cross-fertilized, and most plants set little or no seed when pollinated with their own pollen. The ability to set seed upon selfing has been the subject of many investigations in rye, owing to the importance that this ability will have in an inbreeding program. Attempts have been made to breed self-fertile strains in order to overcome the negative influence on seed set that may be exerted by adverse weather during flowering of the wind-pollinated rye. Most of the investigations have, thus, had a practical aim, and it has been found that plants and varieties may differ in the ability to set seed upon selfing, that these differences are heritable, and that the self seeding ability may be increased by selection. The literature on the subject has been reviewed on several occasions (*e.g.*, HERIBERT NILSSON, 1916; BREWBAKER, 1926; PETERSON, 1934; *cf.* also Imp. Bureau of plant genetics: Rye Breeding Bibliography, 1932). Recent papers on self-fertility in rye were published by HERIBERT NILSSON (1953) and SURIKOV (1956 a and b).

The average of self-seeding is generally somewhere in the range of 1—10 % seed set, the ratio of open/self seed set being about 10—50. The decreased seeding upon selfing has been demonstrated to result from the action of a special incompatibility system (LUNDQVIST, 1954, 1956, 1957, 1958). Two loci, designated S and Z, respectively, each probably with a series of multiple alleles, control the production of certain specificities in the pollen and in the style. Like specificities in pollen and style will cause inhibition of pollen tube growth. Thus the self pollen will automatically become inhibited. The haploid pollen obtains one S and one Z factor, and these two factors autonomously (gametophytically) control the specificity of the pollen grain in question. In some way they co-operate in producing these specificities, for the pollen

will effect fertilization when it has either the S or the Z factor but not both in common with the style, and it will do it as effectively, as if it had no such factor matched in the style.

It was demonstrated that the outlined system was at work in a population of 15 plants of the commercial variety Steel-rye, displaying various degrees of self-seeding upon bagging, the upper level being about 10—15 % (LUNDQVIST, 1954). Thus, there were different degrees of pseudo-compatibility, which means that genetically incompatible pollen may effect fertilization. EAST and PARK (1917) originally coined the term pseudo-fertility for covering the above phenomenon in *Nicotiana*. Factors related to pseudo-compatibility in several species have been thoroughly reviewed and discussed by BRIEGER (1930) and STOUT (1938). The varying degrees of pseudo-compatibility may directly reflect differences between the incompatibility factors themselves, or may result from the action of modifiers, or depend on the plant physiology. Sometimes it may be quite difficult to distinguish between pseudo-compatibility and true compatibility.

It should be observed that the incompatibility system, as it is outlined above for a diploid Angiosperm species, *viz.*, with two loci determining the pollen specificities co-operatively, has so far been found only in grasses, where *Festuca pratensis* (LUNDQVIST, 1955 and unpublished) and *Phalaris coerulescens* (HAYMAN, 1956) appear to react in a similar way. The previous rye investigations by the present author have ultimately aimed at an understanding of the way in which the two loci co-operate, how they are related to one another, and how the two-loci system is related to the one-locus systems at work in other Angiosperms. With these aims the present study of self-seeding and how it is acquired in rye, was initiated. It is evident that knowledge of the genetics of the mechanism, by which otherwise functional pollen is inhibited in the self style, is a prerequisite for an understanding of why this mechanism may fail. It is equally evident that the ways, in which this mechanism may be upset, may give valuable information about the incompatibility mechanism itself.

II. MATERIALS AND METHODS

The present material was obtained exclusively from the commercial variety Steel-rye, where both the diploid and the tetraploid strains have been studied. The diploid strain is derived from the Petkus rye (LJUNG, 1921) and has been bred to a very high degree. The tetraploid strain is

derived after colchicine treatment in 1940 from seven plants and has been subject to rigid selection for increased fertility (MÜNTZING, 1951). Details about the various materials reported here will be given under the respective headings.

All materials that were subject to an analysis of cross-incompatibilities were kept in the greenhouse from the time of flowering an onwards. Most of these plants were grown in pots after transplantation from beds. Materials grown in the field were sown with the use of marking boards and spaced at 10 cm. intervals in rows 15 cm. apart. The tetraploids were not made subject to a control of their chromosome numbers.

For details about the technique in the incompatibility studies, see LUNDQVIST (1954, 1956). Single pergamine bags of the size 5×15 cm. were used in cross-pollinations, whereas for selfings bags with the size of 6×20 cm. were used, single ears being enclosed here. For materials grown in the field double pergamine bags of the size 15×26 cm. were used. One to several ears were enclosed here, depending on the tillering of the plant, and the bag was attached to a stake. Only in the case of the material given in section IV: 1 b below, one or two ears were isolated in single bags, size 6×20 cm., and the bagged ears were left moving freely in the wind. This method was used in order to save time, and the success was highly dependent on the favourable weather that followed, as the single ears might otherwise have become easily damaged by wind and rain occurring together. All isolations were performed one or two days before anthesis of the ear or ears in question. Because of this precaution it is considered from many years' experience that contamination due to stray pollen is insignificant and can be neglected. All self-pollinations occurred spontaneously in the bag, and seed setting has been determined in relation to the total number of flowers isolated. As a rule, only one isolation per plant was made.

The statistical calculations are in accordance with BONNIER and TEDIN (1940 and 1957). SNEDECOR (1946) has been followed in a case of transformation of percentages into angles (angle=arc sin $\sqrt{\text{percentage}}$). The adjusted χ^2_c has been used in cases of two samples and one degree of freedom. Exact probabilities in cases of one sample and one degree of freedom with small expected frequencies have been calculated binomially. With two samples and one degree of freedom, the exact probabilities have been calculated according to BONNIER and TEDIN (1940, pp. 226—227).

III. PATTERNS OF SELF-SEEDING AT THE DIPLOID LEVEL

1. Pseudo-compatibility in relation to incompatibility factors

Of the 15 clones where incompatibilities were studied (LUNDQVIST, 1954), three were investigated in more detail, and No. 21 was rather weakly self-incompatible (LUNDQVIST, 1956). The relatively high degree of pseudo-compatibility did not preclude an exact analysis of the genetics of incompatibilities; on the contrary it was so useful in this connection, as to make the incompatibility investigations concentrate upon that clone. Pseudo-compatibility being infrequent in the two other clones, Nos. 3 and 16, only material derived from clone 21 will be considered here. A definite and complete assignment of genotypes within this material was not attempted, but most of the families obtained from compatible sib matings have in common that they contain both het het and ho het but no ho ho plants. The ho ho plants on the whole being quite rarely obtained, the studies of pseudo-compatibilities will, therefore, be restricted to these families. They were obtained in 1954—1957, the degree of inbreeding by which they were derived gradually increasing from year to year. For further technical data, see LUNDQVIST (1958).

The 19 families are given in Table 1, the het het and the ho het (pooled) categories being considered separately. It may be seen from the totals that self seed set and frequency of self-seeding plants is slowly but steadily decreasing with increasing inbreeding. On the whole, the picture of pseudo-self-compatibility is remarkably similar in the families, self-seeding plants being found in all families, mostly in a clear majority, and self seed setting ranging from 0—20 %.

In 13 of the 19 families the degree of pseudo-self-compatibility is higher in the het het category, the distribution corresponding to $P=0.167$ when equality is expected. It may be seen from Table 1 that this tendency is the same in all years. In Table 2 are given the het het and ho het self seed set distributions, pooled for each of the four years. The tendency being equal for all years, a χ^2 analysis was applied to the grand totals ($\chi^2=13.178$, d.f.$=3$, $P=0.01$—0.001. Also the frequency of pseudo-cross-compatible combinations is higher within the het het group, but the differences are decidedly smaller here, as may be seen from Table 3, and do not reach the level of significance.

The indicated correlation between pseudo-self-compatibility and pseudo-cross-compatibility has been studied in more detail within each of the het het and ho het groups. The primary data for the individual

TABLE 1. *Self seed set in diploid Steel-rye, clone 21. Seed setting in relation to the incompatibility factor genotype.*

(+ denotes at least one kernel set, — denotes complete lack of seed setting.)

Year	Family No.	het het					ho het				
		Total number of seeds/flowers selfed	Total self seed setting in %	Number of plants		Variation range	Total number of seeds/flowers selfed	Total self seed setting in %	Number of plants		Variation range
				+	—				+	—	
1954	17	10/288	3.47	3	1	0— 6.3	13/482	2.70	4	3	0— 8.3
	18	32/416	7.69	6	0	2.9—13.9	34/588	5.78	7	1	0—15.8
	Total	42/704	5.97	9	1		47/1070	4.39	11	4	
1955	11	9/242	3.72	2	2	0—10.0	22/456	4.82	5	1	0—10.5
	12	15/216	6.94	2	1	0—12.8	10/634	1.58	7	2	0— 3.7
	15	3/122	2.46	2	0	1.4— 3.8	0/230	0.00	0	4	0
	16	14/380	3.68	4	0	1.2—12.8	29/714	4.06	5	2	0—20.8
	19	4/68	5.88	1	0	5.9	7/186	3.76	3	0	1.7— 5.6
	20	18/366	4.92	5	0	1.5—10.5	23/436	5.28	6	0	1.3—18.8
	Total	63/1388	4.54	16	3		91/2656	3.43	26	9	
1956	26	61/1108	5.51	12	3	0—17.6	29/558	5.20	6	1	0—11.9
	27	13/432	3.01	6	0	1.1— 5.6	5/280	1.79	3	1	0— 4.1
	28	16/322	4.97	5	0	1.6— 8.3	5/244	2.05	4	0	0— 4.4
	29	6/248	2.42	3	1	0— 6.8	4/254	1.57	3	1	0— 3.0
	30	4/162	2.47	2	1	0— 3.8	14/304	4.61	3	2	0—10.7
	32	33/706	4.67	9	2	0—11.1	4/468	0.85	3	5	0— 3.2
	33	34/646	5.26	8	2	0—10.6	2/426	0.47	2	5	0— 1.9
	34	5/418	1.20	2	3	0— 2.8	13/554	2.35	2	2	0— 8.2
	Total	172/4042	4.26	47	12		76/3088	2.46	26	17	
1957	38	9/578	1.56	5	1	0— 2.3	7/640	1.09	3	3	0— 4.8
	39	18/540	3.33	5	1	0— 8.3	22/2050	1.07	11	7	0— 2.6
	41	22/692	3.18	5	3	0—14.3	33/710	4.65	7	0	1.1—13.6
	Total	49/1810	2.71	15	5		62/3400	1.82	21	10	

TABLE 2. *Material of Table 1 pooled.*

Year	Genotype category	Self seed set in per cent 0	2.5	5	10	25	n
1954	het het	1	5	1	3		10
	ho het	6	4	4	1		15
1955	het het	9	3	3	4		19
	ho het	21	7	4	3		35
1956	het het	22	15	17	5		59
	ho het	29	8	3	3		43
1957	het het	12	5	2	1		20
	ho het	23	5	2	1		31
Pooled	het het	44	28	23	13		108
	ho het	79	24	13	8		124

Difference between het het and ho het in the pooled material: $\chi^2=13.178$; d. f.$=3$; $0.01 > P > 0.001$.

families being sometimes quite meagre (as no complete diallel sib crosses could be performed), they will not be presented here. Only the total, pooled, material from all four years is given in Table 4. Within incompatible combinations, seeding is contrasted with lack of seeding, and the table shows the frequency of seeding sib combinations, when a specific plant category is involved as male or female. The tendency is the same within both the het het and the ho het groups: pseudo-cross-compatibility is higher for the pseudo-self-compatible category, both at the female and the male side. In neither of the four cases does the difference between the self-seeding and the non-self-seeding category reach statistical significance, but it corresponds in all cases to a probability essentially below 0.5. The greatest differences are found within the het het groups (P=0.075 and 0.080).

TABLE 3. *Frequency of seed setting* (+) *and no seed setting* (—) *in incompatible sib combinations within the families of Table 1.*

Mating category	No. of comb. −	+	Per cent + comb.	Heterogeneity between	χ^2_c	d. f.	P
1. het het×het het	141	39	21.7	1 and 3	0.763	1	0.5—0.3
2. het het×ho het	187	49	20.8	2 and 3	0.522	1	0.5—0.3
3. ho het×ho het	138	29	17.4	1 and 2	0.011	1	0.95—0.90

TABLE 4. *Number of seeding (+) and not seeding (—) incompatible cross combinations on the female and the male side, when the involved plant was pseudo-self-compatible (+) or set no self seed (—).*

	Genotype category	Self-seeding category	No. cross combinations		% + in crossing	χ^2_c	d. f.	P
			—	+				
Female side	het het	—	47	7	14.9			0.075
		+	273	79	22.4			
	ho het	—	45	7	13.5			0.231
		+	90	22	19.6			
Male side	het het	—	21	2	8.7			0.080
		+	119	37	23.7			
	ho het	—	88	16	15.4	0.980	1	0.5—0.3
		+	237	61	20.5			

The correlation between pseudo-self-compatibility and pseudo-cross-compatibility is weak, but it seems to be sufficiently strong to indicate that the slight variation in pseudo-self-compatibility is not completely due to chance. At least part of it may be directly due to genetical differences. The differences between seeding and non seeding in incompatible combinations might, thus, be interpreted as merely reflecting general seeding ability, but two objections may be made against this interpretation. (1) It could not be demonstrated that, within the ho het groups, general seeding ability (as calculated from seed setting within compatible sib combinations) is higher for the self-seeding than for the non-self-seeding category. The differences in seed setting are not greater here than may be expected from the rather high variability within the categories (see Table 5). (2) The observed correlation between pseudo-self-compatibility and pseudo-cross-compatibility is equally established at the male side.

Thus, it is probable that the different degrees of pseudo-compatibility are to a great part due to factors which directly affect the reaction between pollen and style. The differences within the ho het group might then be explained from the four different S, Z genotypes within this group. Within the het het group, on the other hand, only one S, Z genotype is obtained. The variation observed here indicates an influence on the incompatibility reaction exerted by genes other than S and Z, and that these genes may be active both in the pollen and in the style.

TABLE 5. *Pseudo-self-compatibility in relation to seed setting of ho het plants when compatibly crossed.*

Year	Family No.	Pseudo-self-compatibles (+)			Setting no self seed (—)		
		Number of plants	♀ compatible combin.		Number of plants	♀ compatible combin.	
			Number	Mean seed set %		Number	Mean seed set %
1954	17	3	9	73.1	3	6	51.7
	18	7	30	71.5	1	3	50.8
1955	12	4	7	44.6	2	3	47.5
	16	5	21	66.3	2	8	55.6
1956	26	6	11	49.8	1	2	45.0
	27	1	1	27.5	1	3	30.8
	28	2	6	40.0	2	4	32.5
	29	3	4	32.5	1	2	67.5
	30	2	2	50.0	2	2	52.5
	32	3	5	42.5	5	9	50.3
	33	2	5	55.5	5	9	33.1
	34	2	9	49.2	2	7	61.1
1957	38	3	6	40.8	3	8	44.4
	39	11	41	64.3	6	25	60.7
Total		54	157	50.5	36	91	48.8

Analysis of variance:

Source of variation	d. f.	Sum of squares	Mean square
a. Between years	3	16587.00	5529.00
b. Within years	244	93730.75	384.25
c. Between families	10	10882.25	1138.25
d. Within families	234	82848.50	354.00
e. Between + and —	14	7931.25	566.50
f. Within + and —	220	74917.25	340.50
g. Between plants	62	28610.75	461.50
h. Within plants	158	46306.50	293.00
Total	247	110317.75	

Variance ratios:

g/h=1.575 P=0.05—0.01
e/g=1.228 P > 0.2

Differences within the groups, in any case the het het group, probably result from varying background genotype. On the other hand, it is not probable that differences between the het het and ho het categories could to any higher degree reflect differences in that background. Differences between the different S and Z genes are indicated, instead. Thereby, the het het group is not unexpected to display the higher degree of pseudo-self-compatibility. In this group all the possible pollen and style specificities, determined by the S and Z genes in question, are tested upon selfing.

2. Population analyses

Since 1945 selfing was practiced in diploid Steel-rye, and the patterns of self-seeding were studied for several years in successive élite populations and their inbred derivatives. In a previous discussion of the way of action of the incompatibility factors in rye, data from 1947—1952 were published to show that self-incompatibility was pronounced in all élite populations studied (LUNDQVIST, 1957). About 95 % of the diploids were found below the limit of 5 % seed setting, and less than one per cent gave a reasonably full seed set upon selfing. Within these limits there were small but clear differences between the various élite populations. In the diploid S_1 generation the frequency of plants highly fertile upon selfing was considerably increased, the high self-fertility being mostly retained upon further inbreeding. Data bearing on the nature and inheritance of low and high self-seeding in the populations studied, will be presented and discussed in full here.

a. Occurrence and degrees of self-seeding in outbred populations

Seed setting upon selfing and open flowering in 1549 plants belonging to the populations of 1946—1950, are summarized in Table 6. It can be seen that, with the exception of the population of 1946, there is practically no overlapping between the self and the open series. Omitting the high-seeding group of this population — which will be discussed in a later section — the equal tendency in self-seeding for the five years is obvious. Differences between the other populations were indicated in a previous statistical analysis (LUNDQVIST, 1957). They become increased, when the self-seeding percentages are transformed to angle values in order to approach towards a normalization of the frequency distributions (Table 7). Most probably they are mainly due to seasonal variations, as there is no reason to suppose that the self-seeding ability is essentially affected by the selection work in rye.

TABLE 6. *Self and open seed setting in élite populations of diploid Steel-rye.*

	Year	Per cent seed setting																						n	Mean
		0	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
Self-pollination	1946	44	140	5	1	1	—	1	—	1	2	1	5	5	4	5	7	3						181	13.77
	1947	139	266	1	—	1	—	—	—	—	—	—	—	—	—	2								270	3.06
	1948	262	628	6	3	2	—	1	1	1	—	—	—	—	1	—	1							644	2.98
	1949	68	154	5	—	1																		160	2.75
	1950	67	272	15	3	1	—	1	—	—	1	1												294	3.28
Open flowering	1946												1	3	1	11	12	44	52	42	15			181	81.01
	1947								3	3	4	1	5	12	10	19	45	44	47	40	28	9		270	72.06
	1948		1	—	—	—	—		1	2	1	—	2	4	8	21	36	71	132	186	129	39	9	642	79.65
	1949		1	—	—	—	—	—	1	3	1	—		5	12	9	24	26	41	21	8	4		156	71.73
	1950									2	1	—		2	2	2	7	17	27	60	89	69	16	294	84.83

TABLE 7. *χ^2 analysis of self seed set in the material, given in Table 6.*

Year	Self seed set transformed to angle values													n
	0	5	10	15	20	25	30	35	40	45	50	55	60	
1947	200	58	9	—	1	—	—	—	—	—	1	1		270
1948	450	161	19	5	3	1	2	1	—	—	1	1		644
1949	98	52	7	2	1									160
1950	141	112	26	9	3	—	1	—	2					294

Classes 10—60 pooled.
χ^2=63.376; d. f.=6; P < 0.001.

The distribution of seed set values upon open flowering in all years is of the normal type with a slight tendency towards negative skewness, the distributions ranging from 25—100 %. No correlation can be found between self (transformed) seed set and general fertility (Table 8). Judging from the strong skewness and the narrow range of the self seed set distribution, these correlation coefficients may be of a rather limited value, but it is interesting to note that the correlation is zero even in 1946, when a relatively high proportion of plants were obtained in the higher self-seeding classes. It may be concluded that in the outbred populations general fertility is only a secondary factor affecting self-seeding ability.

b. Inheritance of self-seeding ability in inbred populations

The parent/offspring relations in self seed setting have been studied in materials of 1946—1949. The first isolation data from 1945 were, for various reasons, less reliable, and beyond the S_3 generation the representativeness of the offspring material generally became too narrowed, the majority of the lines becoming extinct during the continued inbreeding. The material of 1950 is omitted to facilitate the later comparison

TABLE 8. *Correlation between self and open seed set in the material given in Tables 6 and 7.*

Year	n	r	t^2	P
1946	181	0.0452		insignific.
1947	270	— 0.0151		»
1948	642	— 0.0243		»
1949	156	0.1360	2.9027	0.2—0.05
1950	294	0.0047		insignific.

TABLE 9. *Seed setting upon selfing. Parent/offspring relations in Pop/S_1—S_2/S_3 of diploid Steel-rye.*

Parent plants			Offspring plants								
Generation	Years		Plant self seed set in per cent						Per cent plants above 10 % self seed set	Number of	
			0	5	10	25	50	100		plants	families
Pop.	1946—48	0—5	345	13	14	22	20		13.5	414	256
S_1	»		78	3	2	2	6		11.0	91	63
S_2	1947—48		17	4	5	3			27.6	29	17
Pop.	1946—48	5—10	44	5	5	1	1		12.5	56	11
S_1	»		45	8	9	4	2		22.1	68	23
S_2	1947—48		18	3	6	2	1		30.0	30	21
Pop.	1946—48	10—25	20	6	8	7	1		38.1	42	7
S_1	»		56	14	33	35	10		52.7	148	45
S_2	1947—48		17	8	12	25	13		66.7	75	36
Pop.	1946—48	25—50	9	4	17	27	28		84.7	85	8
S_1	»		25	16	54	109	46		83.6	250	75
S_2	1947—48		24	5	39	137	62		89.1	267	68
Pop.	1946—48	50—100	31	16	47	177	250		91.0	521	34
S_1	»		27	14	43	150	113		88.2	347	84
S_2	1947—48		4	2	8	43	35		93.5	92	28

with a corresponding parallel tetraploid material, where the 1950 data became modified through various external influences.

Thus, the following parent/offspring relation categories will be considered: Pop/S_1 with three groups, S_1/S_2 with three, and S_2/S_3 with two groups. The individual parent/offspring data are obtained from two successive years, but judging from the general trend of self seed setting in the élite populations, clear but small differences between them being admitted (*cf.* Tables 6 and 7), such a comparison between seed set data of different years may be justifiable. From the same consideration and in order to save space, the data within the three relation categories have been summarized pooled in Table 9, 2515 plants representing 776 parent lines being presented.

The three categories being unequally represented (no S_3 data for 1947 are available), the data in the table must be judged with some caution. Nevertheless, the tendency is quite clear: offspring from low-seeding plants may cover the whole range of variation in self seed set, as may be the case, when the parent plant was high-seeding, but when the parental self-seeding exceeds the 10 % level, there is a rapid and steady increase of the frequency of high-seeding offspring. In the class with the lowest parental self-seeding, 10—25 % of the offspring exceed the 10 % level of self seed set, whereas the corresponding values for the highest parental class is about 90 %. With continued inbreeding, the frequency of high-seeding offspring for the lower parental classes is increased. This might in part indicate an effect of selection for high self-seeding, but it may also result from parental understatements. That means that the inbred plants may be more sensitive to external agencies, mostly acting negatively on the self-seeding ability, so that the parental plant might not display its true level of self-seeding.

The eight possible parent/offspring correlation coefficients, given in Table 10, are all positive and highly significant. The table shows that a very great part of the variation in self-seeding ability is directly depending on genetical factors. In this connection, the intimate relationship between parent mean square and correlation coefficient should be observed: the narrower the variation range of the parental material, the less pronounced the parent/offspring correlation. This indicates that self seed set variation due to chance does occur, but that the range of this variation is rather narrow.

The eight correlations for self seed set between parent and offspring plants are given in more detail in Table 11. It should be observed here that the table does not present the whole material available, the parental

TABLE 10. *Correlation for self seed set between parental plant and offspring family of diploid Steel-rye.*

Parent/offspr.		No. offspr.		Mean square		r	t^2	P
		plants	fam.	Parent	Offspr.			
Pop/S_1	46/47	604	70	905.1	492.5	0.874	220.136	< 0.001
	47/48	175	74	112.7	210.2	0.301	7.193	0.01
	48/49	339	172	67.8	265.7	0.363	25.853	< 0.001
S_1/S_2	46/47	79	12	747.7	202.1	0.707	10.000	0.01
	47/48	558	165	562.4	340.5	0.672	134.445	< 0.001
	48/49	267	113	506.8	349.7	0.680	95.320	< 0.001
S_2/S_3	47/48	271	51	203.5	216.7	0.449	12.404	0.001
	48/49	222	119	441.9	392.0	0.645	83.342	< 0.001

classes which were not represented by at least three offspring families being omitted. Although the materials are inbred to different degrees and grown in different years, the similar tendency is quite clear. Opposed to the depression effect of inbreeding, resulting in lowered seed setting, is the occurrence of offspring plants with relatively increased self-seeding ability. The chances of obtaining parental understatements are certainly higher in the lower classes of parental seed-setting, but considering the considerable depression effect of inbreeding, obvious in the higher parental classes, it is of interest to note that, up to about 25 % parental seed set, the offspring mean as a rule exceeds its parental class value. Indicated is that the offspring plant with higher self-seeding may sometimes differ in quite a distinct way from its parent. That the higher self-seeding may not always be only a difference in degree is also indicated by the sudden increase of the offspring mean, when the parent exceeds the level of 10 % self seed set. This increase is especially evident, as there is no essential difference in the offspring means between the parental classes 0—5 and 5—10 per cent self set. Obviously there is legitimate reason to oppose to one another self seed setting below and above the 10 % level.

This suggestion is supported from the data in Table 12. Offspring plants with low self-seeding may be obtained both from high- and from low-seeding parent plants. Naturally a limit to the self-seeding ability is given by general seeding ability as measured by open seed set, and the table gives open seed set of offspring plants in the lowest self-seeding class (0—5 %) when they were derived from the low (below 10 %) or

TABLE 11. *Relations in seed setting upon selfing between parent and offspring plants in diploid Steel-rye.*

Offspring means of seed setting in bold types; corresponding numbers of offspring families in thin types.

Offspring		Parental self seed setting in per cent																	
Generation	Year	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
S_1	1947	29 **7.1**	4 **3.8**	—	—	—	—	—	—	—	—	5 **40.0**	6 **39.3**	4 **48.4**	5 **50.6**	7 **50.9**	3 **47.3**		
S_2	»	5 **6.8**																	
S_1	1948	71 **8.0**	—	—	3 **8.2**														
S_2	»	17 **6.6**	13 **9.1**	15 **16.6**	6 **9.1**	11 **20.8**	9 **33.2**	8 **29.8**	11 **25.7**	5 **36.3**	9 **42.7**	16 **40.8**	9 **45.0**	15 **36.2**	6 **36.5**	9 **38.5**	3 **47.5**		
S_3	»			3 **22.5**	4 **25.6**	3 **31.6**	6 **30.7**	9 **38.2**	8 **40.8**	5 **41.4**	4 **38.8**	4 **38.0**							
S_1	1949	156 **8.0**	6 **8.8**	3 **16.6**															
S_2	»	40 **6.9**	10 **6.1**	3 **17.5**	5 **29.6**	4 **28.6**	7 **20.6**	7 **30.8**	4 **38.8**	6 **25.8**	8 **33.9**	7 **38.6**	—	5 **47.5**	—	3 **40.3**			
S_3	»	15 **9.0**	21 **10.7**	8 **14.3**	9 **30.6**	9 **28.7**	7 **24.7**	7 **32.5**	7 **34.6**	12 **32.5**	3 **35.0**	6 **42.5**	4 **36.7**	3 **65.5**	5 **50.5**				
Grand total means		**7.5**	**7.7**	**17.5**	**20.6**	**27.4**	**27.3**	**32.8**	**35.0**	**34.0**	**37.6**	**39.9**	**40.3**	**49.4**	**45.9**	**43.2**	**47.4**		

TABLE 12. *Open seed set in diploid plants with low self-seeding (0—5 %), when the parent plant displayed low or high self-seeding.*

Offspring		Parent self seed set in %	Offspring open seed set in %						No. of offspring		Difference between »low» and »high»			
generation	years		0	5	10	25	50	100	plants	families	χ^2	d. f.	P	Classes pooled
S_1	1947—1949	0—10	10	5	25	84	208		332	213	15.299	2	<0.001	0—25
		10—100	3	4	12	12	29		60	24				
Total			13	9	37	96	237		392	237				
S_2	1947—1949	0—10	2	3	6	28	57		96	55	26.830	2	<0.001	0—25
		10—100	11	8	20	19	28		86	54				
Total			13	11	26	47	85		182	109				
S_3	1948—1949	0—10	2	1	4	8	4		19	15			0.497	0—25;
		10—100	8	—	4	10	7		29	22				25—100
Total			10	1	8	18	11		48	37				

the high (above 10 %) parental self-seeding category. Both for S_1 and S_2 offspring, the proportion of plants with poor open seed set is much higher, when the parent belonged to the category with high self-seeding. In the S_3 generation the difference does not reach statistical significance, but the tendency is the same. Thus, data indicate that, to a high degree, the causes of low offspring self seed set may be different for the two parental categories. Poor self seed set may be effected by a self-incompatibility system or may indicate depression, resulting from inbreeding and blurring the action of factors promoting self seed set.

IV. HIGH AND FULL SELF-SEEDING AT THE DIPLOID LEVEL

Heritable degrees of self-seeding related to the incompatibility loci S and Z, but also determined by other factors, were made probable in the foregoing section. At the same time indications were obtained that there might not always be a difference of degree between low and high self-seeding. A different type of factor was indicated to be at work above the 10 % level of self seed set in diploid rye. In the following, »high» self-seeding will denote a seed set above 10 %, whereas »full» self-seeding is only meant relatively, in comparison to seed set upon open flowering.

1. Genetics of some cases of high self-seeding ability

a. Suddenly appearing high self-seeding ability

Upon selfing in 15 self-incompatible clones of diploid Steel-rye, offspring plants with practically full self-seeding were obtained in three of them (LUNDQVIST, 1954). In two of the clones, Nos. 15 and 17, the high-seeding S_1 plants could be selfed and reciprocally backcrossed to the parent plant and compared to low-seeding sibs in similar backcrosses. The results are summarized in Table 13. Parent clone 15 gave 1.68 % self seed set (four ears with no, 17 ears with 0—5 %, and one with 5—10 % seed set, out of 22 ears selfed in 1952). Eight of the 13 S_1 plants were selfed, and seven of them were high-seeding (range 25.0—63.6 %) with pollen compatible on the parent. Three of the high-seeding plants reacted incompatibly to the parental pollen, four reacted compatibly. Parent clone 17 gave 0.66 % self seed set (9 ears with no, 4 ears with 0—5 % seed set, out of 13 ears selfed in 1952). Seven of the eight S_1 plants were selfed, and one of them set 50 % self seed and reacted compatibly both ways with the parent.

TABLE 13 a. *S_1 family 15. Seed setting in per cent upon selfing and backcrossing in both directions. Data from 1953.*

P=parental clone.

Seed setting in per cent upon	P 15	S_1 plant No.							
		4	6	13	7	10	1	11	5
selfing	1.5	3.5	54.6	25.0	63.6	55.3	48.5	53.7	44.3
$S_1 \times$ P 15		32.4	3.5	4.1	4.4	27.4	47.4	61.1	61.7
P 15 $\times S_1$		0.0	73.8	47.5	62.5	76.3	64.0	65.4	80.2

b. *S_1 family 17. Seed setting in per cent upon selfing and backcrossing in both directions. Data from 1953.*

Seed setting in per cent upon	P 17	S_1 plant No.						
		8	5	1	7	2	4	6
Selfing	0.3	0.0	0.0	0.0	0.0	0.0	0.0	50.0
$S_1 \times$ P 17		0.0	0.0	47.2	47.3	73.8	86.8	20.7
P 17 $\times S_1$		—	0.0	0.0	0.0	4.2	1.2	48.7

The high-seeding plants were, thus, compatible as male in backcrosses to their parent plants, as they were also in all intercrosses to their siblings. In all similar backcrosses previously investigated, low-seeding self offspring were incompatible on their parent plants (LUNDQVIST, 1954). On the other hand, the high-seeding S_1 plants of clone 15 might react incompatibly to all the original pollen specificities capable of being formed by the parent plant (plants 6, 7, and 13). A similar reaction pattern has previously been described, first from *Oenothera* and *Prunus* (LEWIS, 1946, 1949 a and b), and demonstrated to result from a mutation at the incompatibility locus, whereby the previous pollen incompatibility specificity was lost, whereas the action in the style was retained. Such a result indicates that the pollen and the style specificities are controlled separately by a compound incompatibility locus (LEWIS, 1949 b). Two incompatibility loci instead of only one being at work in rye, a continued study of the full self-seeding in rye appeared to be of considerable interest.

The ensuing results obtained from clone 15 turned out rather clear-cut, and the investigation, therefore, concentrated upon this material.

Data from clone 17 were more difficult to interpret and will only be discussed in relation to conclusions arrived at for clone 15.

Self seed set in backcross offspring obtained from $P \times S_1$ combinations, which were compatible both ways, are presented in Table 14, two combinations within clone 15, one within clone 17. Within the clone 15 material, poor pollen production might sometimes blur the border-line between poor seed setting due to lack of pollination and due to true incompatibility. It is quite clear, however, that, both taken absolutely and in relation to open seed set, poor self seed setting in the clone 15 backcross offspring is essentially rarer when the parent plant served as mother. Omitting the doubtful plants, combination $P \times S_1$ in clone 15 gave 35 high-seeding and no low-seeding plants, whereas for $S_1 \times P$ the corresponding numbers were 20 : 13.

It is indicated that part of the pollen of the S_1 plants does not function on the parent plant, and that the only functioning S_1 pollen is that carrying a factor for high self-seeding, the resultant offspring all displaying high self-seeding. The ratio 20 : 13 in the offspring from $S_1 \times P$ might correspond to 1 : 1 ($\chi^2 = 1.484$, $P = 0.3—0.2$), thus suggesting only one heterozygous locus determining this high self-seeding in the S_1 plants 1 and 5. The following experiments were planned from the working hypothesis that this locus might be one of the two incompatibility loci S or Z.

Ten plants from the backcross family S_1 15: $1 \times P$ 15 were taken at random to constitute family 101, and their incompatibility relationships were studied in sib intercrosses. The pollination pattern is given in Fig. 1, and the details of cross and self seed set are summarized in Table 15. There is no difficulty to distinguish between high and low seeding, between compatibility and incompatibility. Five of the plants reacted self-compatibly, their pollen being effective in all combinations tried. Four plants were self-incompatible, and one produced no pollen.

Studying the intercrosses, where self-incompatible plants served as male, it is evident that these plants form two reciprocally compatible groups (plant 5 being put equivalent to plant 3 from later obtained evidence), whereas of the five self-compatible plants three (6, 9, and 10) are exclusively female incompatible, and the other two (1 and 8) may react compatibly or incompatibly. Thus, not more than three groups have to be assumed from the female reactions of the 10 plants, and Fig. 1 gives this »female» grouping. Three groups are expected to obtain, when het het pollinates a related ho het or ho ho plant (*e. g.*, $S_{1.2}Z_{4.4} \times S_{1.2}Z_{3.4} \rightarrow S_{1.1}Z_{3.4} + S_{2.2}Z_{3.4} + 2\ S_{1.2}Z_{3.4}$; and $S_{1.1}Z_{4.4} \times S_{1.2}Z_{3.4} \rightarrow S_{1.1}Z_{3.4} +$

TABLE 14. *Self seed set in diploid reciprocal backcross families, when the backcrossed S_1 plants were self-compatible, the parent plants, self-incompatible.*

	Combination	No. kernels sown	No. mature plants	No. plants tested	Seed setting in per cent Self-pollination 0	10	20	30	40	50	60	70	80	90	100
Parent × S_1	P 15×S_1 15: 1	21	19	19	4[1]	—	—	3	1	5	5	1			
	» ×S_1 15: 5	21	21	21		1[2]	—	—	3	7	7	2	1		
	P 17×S_1 17: 6	21	19	18		1	1	2	2	4	6	2			
S_1×parent	S_1 15: 1×P 15	21	20	10 a)	6[3]	—	—	—	1	1	—	1	1		
				10 b)	5[4]	—	—	—	—	—	1	2	2		
	S_1 15: 5× »	21	19	19	8[5]	—	—	—	1	6	3	1			
	S_1 17: 6×P 17	12	11	11	1	—	—	3	3	2	2				

	Combination	Ratio self/open seed setting in per cent 0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	above
Parent × S_1	P 15×S_1 15: 1	3	1	—	—	—	1	2	3	6	3							
	» ×S_1 15: 5			1	—	—	—	—	7	7	3	1	1	1				
	P 17×S_1 17: 6		1	—	—	1	1	3	1	5	4	—	1	—	—	1		
S_1×parent	S_1 15: 1×P 15	5	—	1	—	—	—	1	—	1	2							
		5	—	—	—	—	—	—	—	1	2	1	—	—	—	—		1[6]
	S_1 15: 5× »	8	—	—	—	—	—	—	3	7	1							
	S_1 17: 6×P 17	1	—	—	—	—	4	—	—	3	1	1	1					

a) Tested outdoors
b) Tested in the greenhouse

[1] Poor pollen producers
[2] Poor pollen producer
[3] 4 plants poor pollen producers
[4] 1 plant poor pollen producer
[5] 1 plant poor pollen producer
[6] ratio 213.9, not reliable

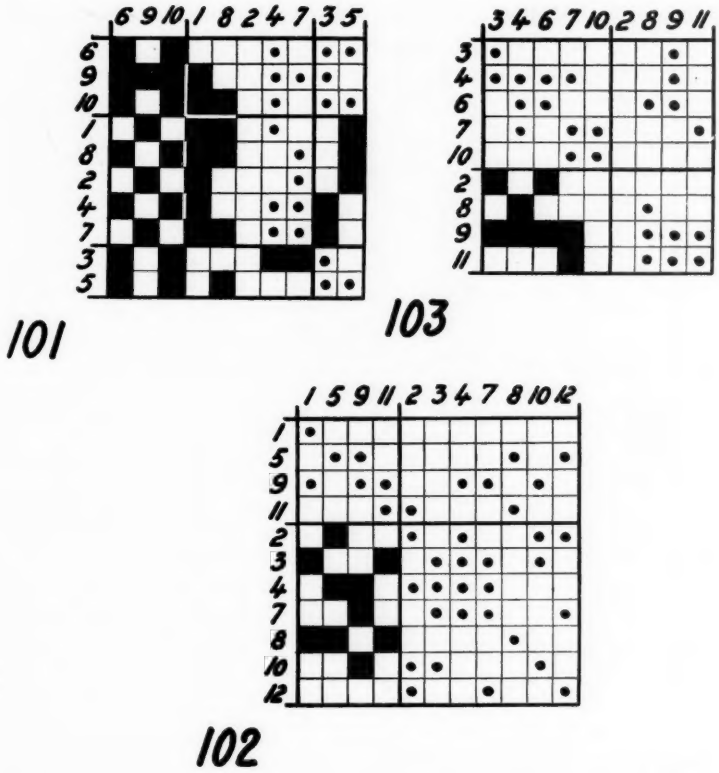

Fig. 1. Results from sib intercrosses within families derived from the self-compatible plant 15: 1. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour.

Symbols:
■ compatible pollination
⊡ incompatible pollination
Blank: pollination not made.
Families derived according to Table 15 a.
For the ambiguous plants 101: 5, 102: 1 and 8, 103: 2, see text.

$+S_{1.2}Z_{4.4}+S_{1.2}Z_{2.4}$, respectively). Thus, data suggest that the self-incompatible parent clone 15 was heterozygous at both S and Z, its genotype being arbitrarily denoted as $S_{1.2}Z_{2.4}$, and that the supposed incompatibility locus mutation did not affect the action in the style but only the pollen specificity. The next question will now be whether the pollinated

TABLE 15 a. *»Self-compatibility» material derived from clone 15.*
S_1+ = self-compatible S_1 plant, ♀ compatible to the parent plant.
BCr+ = self-incompatible, ♀ compatible plant in progeny derived from $S_1 \times P$.

Family No.	Year	Family derivation	Parent Nos.	Number of		
				seeds sown	plants tested	comb. tested
101	1954	$S_1+ \times P$	S_1 15: 1 × P 15	10	10	47
102	1955	BCr+ × BCr+	101: 4 × 101: 3	14	11	36
103	»	»	101: 3 × 101: 4	14	9	24

b. *Seed setting upon selfing and crossing within the »self-compatibility» material of clone 15.* Values classified as incompatible, in bold types.
⊗ = selfing
× = crossing

Family No.	Pollination	Seed setting in per cent											
		0	0	10	20	30	40	50	60	70	80	90	100
101	⊗	**1**	**3**							1	2	2	
	×	**11**	**4**			7	4	3	7	3	7		
102	⊗	**8**	**3**										
	×	**23**	**3** + 1	2	2	2	3						
103	⊗	**6**	**2**										
	×	**14**	**2**	1	1	1	2	2					

S_1 plant 15: 1 was ho het or ho ho (arbitrarily denoted as $S_{1.2}Z_{4.4}$ and $S_{1.1}Z_{4.4}$, respectively).

According to the schemes above, it can be presumed that the two reciprocally compatible but self-incompatible groups in the backcross family are both ho het. If the backcrossed S_1 plant 15: 1 was ho het, these two groups combined will in turn give rise to *three* groups in their offspring (*e. g.*, $S_{1.1}Z_{3.4} \times S_{2.2}Z_{3.4} \rightarrow S_{1.2}Z_{3.3} + S_{1.2}Z_{4.4} + 2\,S_{1.2}Z_{3.4}$). If plant 15:1 was ho ho, correspondingly only *two* groups will be obtained (*e. g.*, $S_{1.1}Z_{3.4} \times S_{1.2}Z_{4.4} \rightarrow S_{1.2}Z_{4.4} + S_{1.2}Z_{3.4}$).

Two such offspring families, Nos. 102 and 103, were analysed; they were derived from the reciprocal cross combination of plants 101: 3 and 101: 4. The pollination patterns of the two families are given in Fig. 1, and seed setting data are summarized in Table 15. Both families display a similar pattern: cross-compatibility is one-way, and two groups may be distinguished. Although the interpretation is not unambiguous due to

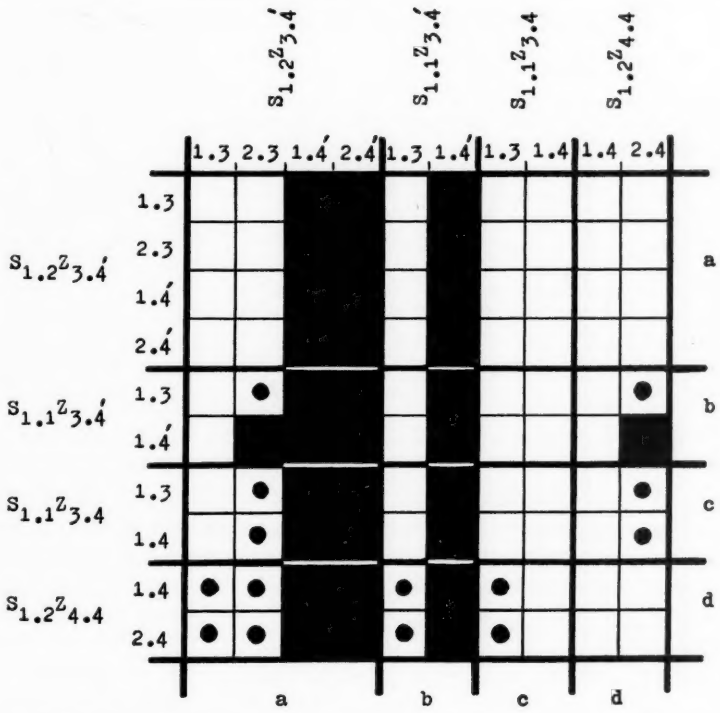

Fig. 2. Reaction pattern upon selfing in progenies obtained from sib intercrosses in family 101. Outside: The postulated zygotic and gametic genotypes of the four groups (a—d) distinguished in family 101. The prime mark denotes the postulated mutated, »self-compatibility», factor at the Z locus.

Symbols:
■ genotype giving self-compatibility
⊡ » » self-incompatibility
Blank: genotype not obtained.

certain gaps in the pollination schemes (the genotypes of plants 1 and 8 in family 102, and of plant 2 in family 103 not being definitely settled), it is not neccessary to assume more than these two groups. Thus, it may be concluded here that the families resulted from a sib intercross ho het × het ho, and that, accordingly, the self-compatible S_1 plant 15: 1 was homozygous at both loci. As above, this plant will be arbitrarily denoted as $S_{1.1}Z_{4.4}$, the self-incompatible parent clone 15 being denoted as $S_{1.2}Z_{3.4}$.

TABLE 16 a. *Progenies from sib intercrosses in family 101. Compatibility segregation when self-compatible sibs were used as male.*

Combination category	Plants inter-crossed	No. of progeny plants							Self-comp. in %	
		Kernels sown	Unclassified			Classified			Observed	Expected
			Total	♂-sterile	not isolated	Total	self-comp.	self-incomp.		
a×a	6×10	16	2	1	1	13	13	—	100.0	100.0
	9× 6	40	3	2	1	31	31	—	100.0	»
	9×10	40	2	—	2	32	32	—	100.0	»
	10× 6	32	3	2	1	24	24	—	100.0	»
b×a	1× 9	24	1	1	—	18	10	8	55.6	83.3
	8× 6	22	1	—	1	18	17	1	94.4	»
	8×10	16	—	—	—	13	12	1	92.3	»
c×a	2× 9	31	2	2	—	15	13	2	86.7	66.7
	4× 6	14	1	1	—	8	6	2	75.0	»
	4×10	24	—	—	—	19	10	9	52.6	»
	7× 9	40	1	—	1	31	23	8	74.2	»
d×a	3× 6	15	—	—	—	11	7	4	63.6	50.0
	3×10	24	2	2	—	19	6	13	31.6	»
	5× 6	32	—	—	—	21	10	11	47.6	»
	5×10	32	—	—	—	26	13	13	50.0	»
a×b	9× 1	16	2	2	—	12	12	—	100.0	100.0
	10× 1	32	1	1	—	29	29	—	100.0	»
	10× 8	32	—	—	—	29	29	—	100.0	»
b×b	1× 8	24	2	2	—	16	16	—	100.0	100.0
	8× 1	8	3	—	3	4	4	—	100.0	»
c×b	2× 1	23	5	5	—	11	11	—	100.0	100.0
	4× 1	16	1	1	—	8	8	—	100.0	»
	7× 1	32	4	4	—	17	17	—	100.0	»
	7× 8	40	—	—	—	36	36	—	100.0	»
d×b	5× 8	40	2	—	2	19	8	11	42.1	50.0

16 b. χ^2 *analysis of compatibility segregation. Pooled data.*

S.c. = self-compatible
S.inc. = self-incompatible

	No. of progeny plants			Self-comp. in %		χ^2	d. f.	P
	Total	S. c.	S. inc.	Observed	Expected			
a×a	100	100	—	100.0	100.0			1.000
b×a	49	39	10	79.6	83.3			0.565
c×a	73	52	21	71.2	66.7	0.685	1	0.5—0.3
d×a	77	36	41	46.8	50.0	0.324	1	0.7—0.5
a×b	70	70	—	100.0	100.0			1.000
b×b	20	20	—	100.0	100.0			1.000
c×b	72	72	—	100.0	100.0			1.000
d×b	19	8	11	42.1	50.0			0.648

Assuming the self-compatibility of S_1 plant 15: 1 to result from a mutation directly involving one of the incompatibility loci S or Z, this plant may, thus, be denoted as $S_{1.1}Z_{4.4}'$, the prime sign denoting a mutant allele which does not any longer determine the pollen specificity previously characteristic of it. Of the six genotypes obtainable from the backcross combination $S_{1.1}Z_{4.4}' \times S_{1.2}Z_{3.4}$, three will have the Z_4' allele giving rise to uninhibited pollen and self-compatible plants, the other three (ho het, het ho, and het het), with the original Z_4 allele, resulting in self-incompatibility.

In the backcross family 101 three of the plants are self-compatible but female incompatible with the self-incompatible sibs and must be judged $S_{1.2}Z_{3.4}'$ (group *a*). The five self-incompatible plants formed two inter-compatible mating groups (*c* and *d*, respectively), and should accordingly be classified as $S_{1.1}Z_{3.4}$ and $S_{1.2}Z_{4.4}$. The two remaining plants (group *b*) were self-compatible and female incompatible with one of the two ho het groups, the genotype presumably being either $S_{1.1}Z_{3.4}'$ or $S_{1.2}Z_{4.4}'$. The ensuing considerations will be evident from Fig. 2.

When group *a* pollinates group *c* ($S_{1.1}Z_{3.4}$), pollen carrying the factors S_1Z_3 will be inhibited, whereas the other pollen genotypes will function and result in $^2/_3$ of the offspring becoming self-compatible, the remaining $^1/_3$, self-incompatible. When group *d* ($S_{1.2}Z_{4.4}$) is correspondingly pollinated all four pollen genotypes from group *a* will function, $^1/_2$ of the offspring becoming self-compatible. Pollen from group *c* does not function on group *b*, so the latter will be denoted as $S_{1.1}Z_{3.4}'$. There remain several other combinations between the four sibling groups, and the

proportions of self-compatible plants in the offspring are readily obtained from Fig. 2.

Offspring from the sib combinations in which self-compatible plants of family 101 served as male, were grown in the field in order to test their self reaction, the results being entered in Table 16. Out of 665 kernels sown, 518 mature plants were obtained and 480 of them self tested in 1955. The poor germination was due to bad weather at the time of sowing, and it is not likely to have seriously reduced the representativeness of the sample. As may be seen from the table, there is excellent agreement with expectation in all eight combination groups. Four of them contain both self-compatibles and self-incompatibles. Pooling the data from these segregating families, 135 self-compatible and 83 self-incompatible plants were obtained, the expected ratio being 137.5/80.5. Thus, it is evident that self-compatibility in clone 15 resulted from a mutation at one of the incompatibility loci (arbitrarily the Z locus), eliminating the pollen specificity previously determined by it but leaving the style action unaffected. Data indicate that this mutation does not reduce the functioning ability of pollen carrying it, when compared with normal pollen in compatible combinations. From the results in Table 14, when the self-compatible mutant S_1 plant of clone 15 was pollinated by the parent in a backcross, it is equally evident that the mutant factor does not impair the viability of the self-compatible plants. Only S_1 plant 15: 1 was analysed in detail, but it is considered most probable that the seven self-compatible S_1 plants from clone 15 all resulted as a cluster from one mutated Z allele.

The results of clone 17 do not conform to this clear-cut scheme. As may be seen from Table 14, the backcross P 17$\times S_1$ plant 17: 6 resulted, as expected, in self-compatible offspring only. In the reciprocal backcross, however, where half of the offspring plants should be expected to be self-incompatible, only one out of 11 plants obtained was self-incompatible. It may be suggested that self-compatibility resulted here in the same way as demonstrated for clone 15, but that some lethal factor linked to one of the incompatibility loci effected an elimination of most of the self-incompatible segregants in this backcross family of clone 17. For instance $S_{1.2}Z^{+}_{4.4}\times S_{1.2}Z^{+}_{3.4}$ (the + sign denoting a linked lethal factor) will give no self-incompatible offspring, save for a limited number resulting from crossing over.

b. The effect of selection for high self-seeding ability

It is evident that self-compatibility may be effected by a single mutant incompatibility factor at the Z locus, but it is not known whether the S locus also is capable of affecting the self-incompatibility in that way. Equally, it may be asked, whether high self-seeding may result from the combined action of several minor factors, which are not located at the S or Z loci. For an attack on these problems an excellent material was available by courtesy of Professor ARNE MÜNTZING: a great number of inbred lines of diploid Steel-rye, obtained after about thirty generations of selfing, most of them being as uniform and constant as pure lines and displaying a very high degree of self-seeding after long-continued selection (*cf.* MÜNTZING, 1943).

Seven of these lines, displaying quite clear morphological differentiation, were diallely crossed, 18 of the 21 F_1 lines possible being obtained. Both the seven inbred lines and their 18 F_1 lines were highly self-seeding (LUNDQVIST, unpublished). They were then crossed to clearly self-incompatible plants from a Steel-rye population. In order to avoid, as far as possible, disturbances of the gametic ratios in the inbred and F_1 material, these plants were pollinated by the selected population plants, pollen from only one plant being used at each time. In all, three different population plants were used on the material of inbred lines, and two other plants on the F_1 lines. The resultant progeny plants were grown in the field in order to test their self reaction, the details of the different offspring materials and the way in which they segregated for low and high self-seeding being entered in Table 17.

In all, 746 plants are entered in the table, for which data from both self and open seed set were available, 5.7 % of the mature plants thus being omitted, for which either data were lacking. Most of the plants gave a practically full seed set upon selfing, the slightly lower seeding in comparison to open seed set being readily explained as an effect of inbreeding. Other plants gave a decidedly low self seed set, some of the cases probably resulting from various accidents or low general seeding ability. Of the remaining plants, for which no such reason of the low self-seeding could be found, and for which true self-incompatibility may obtain, one is found in the Inbred lines$\times$Pop, and 62 in the $F_1\times$Pop offspring material, which corresponds to 0.4 % and 12.9 % of the total number, respectively. It is considered that the single case in the Inbred$\times$Pop progeny is accidental, and that true self-incompatibility may obtain only in the offspring from $F_1\times$Pop.

Admittedly, different population plants were used as pollinators in

TABLE 17. *Self seed set in progeny obtained from the combination of diploid self-compatible inbred lines and their F_1 lines pollinated by self-incompatible population plants.*

Parent Nos.	No. kernels sown	No. mature plants	No. plants tested	Self-pollination 0	10	20	30	40	50	60	70	80	90	100	Ratio self/open seed setting in per cent 0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
19×Pop 4, 5, 6	60	59	53	1[1]	—	1[2]	2	2	4	7	19	14	3		1	—	—	1	1	1	3	5	6	22	10	2	1			
20×Pop 4, 6	39	37	29			1[3]	—	—	4	9	8	6	1					1	—	1	1	4	7	11	4					
21×Pop 4, 5, 6	60	51	49	1[9]	—	—	1	—	1	9	21	15	1			1	—	—	—	—	1	2	13	20	10	1	1			
22×Pop 4, 5	40	24	23	1[10]	—	—	—	—	—	1	6	13	2		1	—	—	—	—	—	—	—	8	5	8	1				
23×Pop 4, 5, 6	49	45	42		1[4]	—	1[5]	—	1	2	17	15	5			1	—	1	—	—	1	2	14	12	10	1				
24×Pop 5, 6	37	35	34	1[11]	—	—	—	1	—	7	16	8	1					1	—	—	—	5	14	8	5	1				
25×Pop 4, 6	40	40	36		1[12]	—	1	2	—	1	8	22	1			1	—	—	2	—	—	2	13	12	6					
Total	325	291	266																											
(19×20)×Pop 1	30	29	26					2	—	5	3	11	5							1	2	1	6	10	4	1	1			
(19×21)×Pop 1	30	29	27				1[6]	—	—	—	7	14	5					1	—	—	—	2	4	11	8	1				
(19×23)×Pop 1a	30	28	28					1	—	2	10	11	4							1	—	3	11	9	4					
(19×24)×Pop 1b	30	30	28				1	2	1	5	12	7								3	1	1	11	8	3	—	—	—	1
(19×25)×Pop 2	30	26	25			1[13]	—	—	1	4	7	8	4				1	—	—	—	1	2	8	6	7					
(20×21)×Pop 1a	30	28	25	5	1	—	—	1	—	—	6	10	2		5	1	—	—	1	—	—	—	5	9	4					
(20×23)×Pop 1b	30	28	26		1[7]	—	1[8]	—	2	4	8	8	2			1	—	—	1	—	2	3	9	8	2					
(20×24)×Pop 2a, b	30	29	29						1	5	13	9	1									4	8	14	2	1				
(20×25)×Pop 1b	30	25	25	5	1	—	—	1	2	1	8	5	2		5	1	—	—	—	1	2	1	3	9	3					
(21×23)×Pop 2b	30	23	23	5	1	—	—	—	—	1	4	10	2		5	1	—	—	—	—	—	—	7	6	4					
(21×24)×Pop 2	30	26	25	6	—	—	—	—	—	4	3	11	1		6	—	—	—	—	—	—	4	3	11	1					
(21×25)×Pop 1	30	29	28				2	—	2	1	5	14	4					1	1	1	—	1	6	13	5					
(22×23)×Pop 1a	30	28	27	7	2	—	—	—	—	2	3	10	3		7	1	1	—	—	—	—	1	1	15	1					
(22×24)×Pop 1	30	29	29	5	1	1	—	—	1	—	9	11	1		5	1	1	—	—	—	—	—	7	13	2					
(22×25)×Pop 1	30	28	27						1	1	3	20	2									2	3	18	4					
(23×24)×Pop 2a	30	29	29					1	—	10	13	5									1	5	14	7	2					
(23×25)×Pop 1a	30	26	24	9	4	—	—	—	—	—	—	10	1		8	5	—	—	—	—	—	—	4	6	1					
(24×25)×Pop 1	30	30	29	5	1	1	1	—	—	4	3	13	1		5	1	1	1	—	—	—	4	4	8	3	1	1			
Total	540	500	480																											

Poor self-seeding resulting from:

a. *accidents*

[1] Straw broken
[2] » » down on the ground
[3] Kernels concentrated to the base of the ear
[4] Straw broken
[5] Straw broken
[6] Kernels concentrated to the base of the ear
[7] Straw broken
[8] Kernels concentrated to the base of the ear

b. *low general seeding ability*

[9] Open seed set 16.2 %
[10] » » » 20.5 »
[11] » » » 18.8 »

c. *no obvious reason*

[12] Open seed set 80.4 %
[13] » » » 88.9 »

TABLE 18. *Frequency of self-incompatible offspring plants when seven inbred lines and 18 F_1 lines were pollinated by self-incompatible population plants of diploid rye.*

Progeny families from combination	Not segregating			Segregating		
	Number	Pooled No. of plants		Number	Pooled No. of plants	
		S.-comp.	S.-incomp.		S.-comp.	S.-incomp.
I×Pop	7	266	—	—	—	—
F_1×Pop	10	273	—	8	146	61

the Inbred and F_1 series. Taking the data as they stand, it is found that from F_1 lines involving inbred line 19 no self-incompatible offspring plants were obtained, when self-incompatible Pop pollinated. All the other six inbred lines may be involved in F_1×Pop combinations, where self-incompatible plants were segregating, eight of the 18 F_1×Pop offspring families segregating in that way (Table 18). Evidently the six inbred lines fall into two groups (20, 23, and 24; and 21, 22, and 25, respectively), segregation only being obtained when the F_1 line involved lines from both these groups.

The details of the segregating offspring families are given in Table 19. On the whole, the segregation ratios conform quite well to one fourth of the plants reacting self-incompatibly. The number of plants in the individual families being rather low, the heterogeneity test must be

TABLE 19. χ^2 *analysis of segregating families in Table 18. Expected ratio 3 self-compatible : 1 self-incompatible plant.*

F_1 number	No. of plants		χ^2	d. f.	P
	S.-comp.	S.-incomp.			
20×21	19	6	0.01	1	0.95—0.90
20×25	19	6	0.01	1	0.95—0.90
21×23	17	6	0.01	1	0.95—0.90
21×24	19	6	0.01	1	0.95—0.90
22×23	18	9	1.00	1	0.50—0.30
22×24	22	7	0.01	1	0.95—0.90
23×25	11	13	10.89	1	> 0.001
24×25	21	8	0.11	1	0.80—0.70
Pooled data	146	61	2.20	1	0.20—0.10
Heterogeneity			9.85	7	0.20—0.10

judged with caution. The slight excess of self-incompatible plants in the pooled material is mainly due to the deviating offspring from F_1 line 23×25 and is not unexpected in view of the many environmental factors, which may cause an impairment of self-seeding. In fact, it was noted that two of the 13 low-seeding plants in this family were poor pollen producers, and that the plants might not display true self-incompatibility. It is concluded that all the eight families in question segregated for self-incompatibility according to the same scheme.

This simple scheme implies that a low number of major factors are involved in the segregation of low-seeding plants in the present material. Evidently, self-incompatibility may be effected by more than one locus. The self-compatible inbred lines reacted uniformly upon pollination by a self-incompatible plant, giving only self-compatible offspring, and must be presumed to be homozygous at these loci. The self-compatible F_1 lines did not react uniformly, self-incompatible plants segregating in some of the corresponding offspring families. Heterozygosity may evidently obtain at these loci in some of the F_1 lines.

The data are readily explained if one assumes two independently segregating loci with self-compatibility factors, the pollen carrying at least one such factor at either locus being able to grow down the self style; the inbred lines falling into three groups, lines 20, 23, and 24 being homozygous for a self-compatibility factor at one locus, lines 21, 22, and 25 at the other, and line 19 at both loci. The self-incompatible population plants must be presumed to be devoid of such factors.

Two self-compatibility factors at the same locus will evidently be obtained in the F_1, whenever line 19 is one of the parents, or when the combined lines belong to the same group. Only one factor at each locus will be carried in the F_1, when the combined inbred lines (except 19) belong to different groups. In the former case all the F_1 gametes will carry at least one factor for self-compatibility, whereas in the latter case one fourth of the gametes will carry none. After fertilization by pollen from a self-incompatible population plant, one fourth self-incompatible plants will accordingly segregate here in the offspring.

These factors for self-compatibility act in a way which clearly points to a mutant origin. Their effect being an elimination of the inhibition of pollen tube growth in the self style, there has been a strong selection for them in the course of inbreeding. Evidently, two loci are mainly concerned in these mutations. Although the direct proof is lacking, the conclusion most readily at hand would appear to be that these two loci are identical to the incompatibility loci S and Z. Both S and Z would,

TABLE 20. *Self-seeding in plants classed as self-incompatible in Table 19.*

Self-seeding	F_1 number								Total
	20×21	20×25	21×23	21×24	22×23	22×24	23×25	24×25	
Plants without kernels	—	—	1	1	1	1	—	2	6
Plants with kernels	6	6	5	5	8	6	13	6	55

thus, be capable of effecting self-compatibility in the way previously demonstrated for the Z locus, the mutant factor four times being associated with one of the loci, four times with the other. The occurrence of mutant factors at both loci in inbred line 19 would appear to have a low probability, but may have resulted from a contamination somewhere in the process of selfing. Special experiments are designed to settle how the two loci are related to the S and Z loci.

In the presence of these two loci, each at once effecting practically full seeding upon selfing, the selection pressure for minor factors promoting self-seeding would appear to be low. Data from self-seeding in plants classed as self-incompatible and obtained in the above segregating offspring families derived from the cross self-compatible F_1×self-incompatible Pop, indicate that, in comparison to ordinary population material (*cf.* LUNDQVIST, 1954, Table 1, and 1956, Table 2), the self-incompatible reaction may be less rigorous here (Table 20). Out of 61 such plants selfed, 55 set at least one seed, a result equivalent to the weaker degrees of self-incompatibility found in the population material in question. No adequate control material being available, however, the data have to be judged with caution. In any case, selection for weak S and Z factors or minor factors effecting weak degrees of self-incompatibility could have been effective at an early stage in the course of inbreeding, when mutations to self-compatibility were only rarely present.

2. Occurrence and inheritance of high self-seeding ability in outbred and inbred populations

Data in section IV: 1 pointed to the view that the high and full seed setting upon selfing in diploid Steel-rye were predominantly effected by mutations, which probably directly involved the incompatibility loci S and Z. Variations in the rigour of the self-incompatibility reaction due to minor factors were made probable, but these factors seemed to be

relatively unimportant in effecting high self-seeding in the material in question. In view of the rather limited material investigated, the factors related to high self-seeding have been submitted to a further analysis in the populations of Steel-rye studied in 1946—1949.

The mutant factors for self-compatibility evidently acted in the way that pollen was capable of growing down the self style, when it carried at least one such factor. This explains why high-seeding plants were so frequent in the population of 1946 (see Table 6). The material of kernels from which this population series was raised, was obtained from plants in standard plots, grown in a large group of the aforementioned high-seeding inbred material of Steel-rye, and frequent inter-pollination must have taken place. The pollen without such factors reacting self-incompatibly, such self-compatible plants should be expected to breed true for self-compatibility. Data presented in Table 16 corroborated this conclusion, but it was obvious that inbreeding depression might render the results less clear-cut.

It was evident from Table 9 that self offspring plants with low self-seeding might be obtained in low frequency from high-seeding parents in the populations studied. It may be questioned whether this may indicate that high self-seeding is brought about in a way different to that outlined above, or whether the low-seeding plants are due to disturbances resulting from inbreeding. In order to eliminate unnecessary variables from the problem, only offspring derived from the highest self-seeding parent group (above 25 % self set), will be considered here.

Offspring families belonging to S_1—S_3 generations and grown in 1947—1949, are presented in Table 21, three S_1, three S_2, and two S_3 generations being obtained. Families with at least one low-seeding plant (below 5 % self set) are obtained in proportions which do not seem to follow a clear-cut scheme, but the material does not lend itself to a statistical analysis. The proportion of low-seeding plants within the »low-seeding» families is clearly increased with the degree of inbreeding, 12.4 %, 29.9 %, and 30.4 % of the plants being low-seeding in the pooled S_1, S_2, and S_3 materials, respectively ($\chi^2=28.138$, d. f.$=2$, $P<0.001$). It is interesting in this connection to note that, taking data as they stand, the proportions of »low-seeding» families vary in the opposite direction, the proportions being 45.2 %, 23.9 %, and 20.8 % of the S_1, S_2, and S_3 families, respectively. Such results should be expected from the action of two opposite forces: inbreeding impairment of seed setting ability, and the natural selection of the highest seeding lines in the course of inbreeding.

TABLE 21. *Seed setting in families obtained from highly self-seeding parent plants (above 25 % self set) and containing plants with low self-seeding (0—5 % self set).*

Parent/offspring		No. of offspring families		Seed setting in per cent in offspring plants upon																	
				Self-pollination						Open flowering in plants with											
										self seed set below 5 % (»low«)						self seed set above 5 % (»high«)					
		totally	with low-seeding plants	0	5	10	25	50	100	0	5	10	25	50	100	0	5	10	25	50	100
Pop/S_1	46/47	35	15	34	14	17	68	120		3	5	9	4	12				8	39	163	
	47/48	2	2	3	1	13	25	14						3				2	7	38	
	48/49	5	2	3	1	3	5	2					1	2						11	
Total		42	19	40	16	33	98	136		3	5	9	5	17				10	46	212	
S_1/S_2	46/47	6	4	6	3	10	14	1			3	2	—	1		1	1	2	9	11	
	47/48	102	25	37	9	10	34	16		1	3	11	5	14				4	12	47	
	48/49	51	9	9	2	8	8	7		1	—	1	1	3				1	7	13	
Total		159	38	52	14	28	56	24		2	6	14	6	18		1	1	7	28	71	
S_2/S_3	47/48	39	13	18	1	5	43	11		4	—	1	5	1				4	18	25	
	48/49	57	7	10	—	3	1					1	1	6					1	2	
Total		96	20	28	1	8	44	11		4	—	2	6	7				4	19	27	

Difference in open seed set between »low-seeding« and »high-seeding« groups.

Pooled data.

Parent/offspring	P	Classes pooled
Pop/S_1	< 0.001	0—25; 25—100
S_1/S_2	< 0.001	» »
S_2/S_3	0.022	» »

TABLE 22. *Frequency of high self-seeding in S_1 offspring from diploid population plants with less than 5 % self seed set.*

	Pop/S_1			
	1946/1947	1947/1948	1948/1949	1949/1950
a. $\frac{\text{No. self kernels}}{\text{No. selfed florets}}$ in Pop	315/35763	295/52882	1084/183680	202/27174
b. $\frac{\text{No. »high» plants}}{\text{No. plants, totally}}$ in S_1	5/35	16/114	35/265	20/131
a×b. Rate of »high» S_1 plants	0.00126	0.00078	0.00078	0.00113

A clear indication of inbreeding disturbances resulting in low self-seeding is, thus, obtained, and general seeding ability is therefore studied in the plants of the »low-seeding» families (Table 21). It is found that open seed set is very clearly inferior in the low-seeding group of plants. It is concluded that a considerable part of the plants with poor self-seeding are due to reduced general seeding ability. Considering the several ways in which poor self-seeding may be attained in the absence of true self-incompatibility (*e. g.*, lack of pollen, bagging accidents, lethal factors), truly self-incompatible plants in the offspring from the self-compatible plants in question may be regarded to be rare events. It is concluded that they do not invalidate the hypothesis that, also in the present populations, high and full self-seeding are predominantly effected by mutant factors acting in the way outlined above.

The frequencies in which high-seeding plants were obtained after selfing of nearly 300000 florets of 1188 clearly self-incompatible plants from the outbred populations of 1946—1949, are given in Table 22. High-seeding offspring plants were obtained at a rate varying from 7.8—12.6 per ten thousand selfed florets, the frequencies for the different years, thus, being of the same order of magnitude. No direct calculation of the number of pollen grains per pollinated floret is available, but the number may be estimated to vary from 100—10000, and the rate of self-compatible mutant pollen grains would, thus, lie somewhere between 10^{-5}—10^{-7}. It should be observed that this figure constitutes a maximum estimate of the mutation rate, as at least two loci are capable of these mutations, and part of the mutations may have arisen at some stage before meiosis. The error that may result from misclassified high-seeding plants is considered to be relatively unimportant.

V. PATTERNS OF SELF-SEEDING AT THE TETRAPLOID LEVEL

Parallel to the studies in diploid Steel-rye, selfing was practiced in its tetraploid strain in successive élite populations and their inbred derivatives. Data from 1947—1952 previously published (LUNDQVIST, 1957) demonstrated that the tetraploid strain is strongly self-incompatible in all élite populations studied, about 80—85 % of the plants being found below the limit of 5 % seed setting, and practically no plant giving a reasonably full seed set upon selfing. Indications were obtained that self seed set in the populations was more variable at the tetraploid level. Plants highly fertile upon selfing were equally rare in the S_1 generation. Data, bearing on these differences in self-seeding at the diploid and tetraploid levels, will be presented and discussed in full here.

1. Occurrence and degrees of self-seeding in outbred populations

Self and open seed set in 1510 plants belonging to the tetraploid élite populations of 1946—1950, are given in Table 23. The self series range from 0—60 % seed set, with a pronounced skewness towards low seed set. The differences between the population series, indicated in the previous statistical analysis (LUNDQVIST, 1957), become increased after transforming the self seed set percentages to angle values in order to approach towards a normalization of the frequency distributions (Table 24). The population of 1950 is deviating to a very high degree, the mean for self set being raised here to 12.2 %, against 3.5—4.5 % in the other populations. It was concluded (LUNDQVIST, 1957) that this was due to external influences (low temperature during flowering), and that the incompatibility reaction at the tetraploid level might be less effectively buffered against such influences.

Open seed set has in all years a distribution of the normal type. Although the distribution ranges from 0—95 %, there is only slight overlapping between the self and open series. Only in 1946 is there a slight correlation between self (transformed) seed set and general seeding ability (Table 25). It is of especial interest that the correlation is zero even in 1950, where a relatively high proportion of the plants were obtained in the higher self-seeding classes and the self series approached fairly well a normal distribution. As was found for the diploids, general seeding ability seems to be only a secondary factor affecting self-seeding ability in the tetraploid outbred populations.

TABLE 23. *Self and open seed setting in élite populations of tetraploid Steel-rye.*

	Year	Percent seed setting																					n	Mean
		0	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
Self-pollination	1946	40	158	12	8	4	3	1	1															187	4.18
	1947	101	224	24	9	5	3	3	1															269	4.17
	1948	80	401	44	11	5																		461	3.38
	1949	71	207	24	6	5	1	1																244	3.73
	1950	8	113	79	54	31	25	19	9	8	8	2	—	1										349	12.20
Open flowering	1946				1	—	1	1	3	7	11	21	27	36	38	25	8	4	4					187	56.99
	1947						3	1	6	7	6	12	28	46	55	39	34	19	8	3	1			268	61.62
	1948		1			1	2	—	1	6	3	14	26	55	96	91	95	52	11	2	1			457	65.38
	1949								1	—	1	3	13	26	29	52	46	38	20	9	1			239	69.15
	1950							1	2	3	3	3	8	8	53	56	61	95	42	13	1			349	71.43

TABLE 24. χ^2 *analysis of self seed set in the material given in Table 23.*

Year	Self seed set transformed to angle values											n
	0	5	10	15	20	25	30	35	40	45	50	
1946	79	69	15	10	7	5	2					187
1947	117	94	25	15	9	5	3	1				269
1948	167	189	72	23	10							461
1949	98	88	36	13	7	1	1					244
1950	21	60	68	65	56	32	22	17	7	1		349

Classes 20—50 pooled. For the whole material $\chi^2=506.653$, d. f.$=16$, $P < 0.001$. Excluding 1950, $\chi^2=27.194$, d. f.$=12$, $0.01 > P > 0.001$.

TABLE 25. *Correlation between self and open seed set in the material given in Tables 23 and 24.*

Year	n	r	t^2	P
1946	187	0.2000	7.728	0.01
1947	268	— 0.0139		insignific.
1948	457	0.0076		»
1949	239	— 0.0253		»
1950	349	0.0485		»

2. Inheritance of self-seeding ability in inbred populations

Judging from the general trend of self seed set in the tetraploid élite populations (Tables 23 and 24), and omitting data from 1950, which became modified and deviating through various external influences, it has been considered justifiable to correlate self-seeding data from different years. As was the case for the diploids, tetraploid S_1—S_3 materials from 1946—1949 will be studied in relation to the parental generation, grown in the preceding year. Also here the parent/offspring relation categories will, thus, be unequally represented, and there remain three groups within the Pop/S_1 relation category, three within the S_1/S_2, and two within the S_2/S_3 category. The data within each of the three relation categories are pooled in Table 26, a number of 2494 plants representing 973 parent lines being presented.

As was the case in the diploid material, offspring covering practically the same range of variation in self seed set were obtained for all parental self seed set classes. Also here, with increased parental self-seeding, there is a gradual increase of the proportion of high-seeding offspring plants, but the increase is considerably slower, 5—10 % high-seeding plants being obtained for the lowest class, and 30—50 % for the class of 25—50 % parental seed setting. As is the case in the diploids, the frequency of high-seeding offspring plants is increased with continued inbreeding. Considering the increased resistance to inbreeding at the tetraploid level, an effect of selection for high self-seeding ability might appear somewhat more probable here than in the diploids.

In two respects the tetraploid data definitely differ from the diploid data: (1) Plants with more than 50 % self set are very rare. This might in part be explained from the decreased general seeding ability of the tetraploid strain, were it not for (2) the pronounced skewness towards low offspring self-seeding, observable for all parental classes (except

TABLE 26. *Seed setting upon selfing. Parent/offspring relations in Pop/S_1—S_2/S_3 of tetraploid Steel-rye.*

Parent plants			Offspring plants								
Generation	Years		Plant self seed set in per cent						Per cent plants above 10 % self seed set	Number of	
			0	5	10	25	50	100		plants	families
Pop	1946—48	0—5	459	39	18	4	1		4.4	521	296
S_1	»		202	29	13	2			6.1	246	163
S_2	1947—48		82	11	12	2	1		13.9	108	68
Pop	1946—48	5—10	174	32	21	1			9.6	228	72
S_1	»		156	42	37	10	1		19.5	246	99
S_2	1947—48		55	14	28	6	1		33.7	104	48
Pop	1946—48	10—25	145	57	47	12			22.6	261	44
S_1	»		146	66	72	19	2		30.5	305	82
S_2	1947—48		78	35	38	12			30.7	163	57
Pop	1946—48	25—50	26	20	26	7	1		42.5	80	7
S_1	»		24	23	32	20	1		53.0	100	17
S_2	1947—48		47	36	31	5	1		30.8	120	17
Pop	1946—48	50—100	—	—	—	—	—				
S_1	»		2	—	3	4			77.8	9	1
S_2	1947—48					3			100.0	3	2

TABLE 27. *Correlation for self seed set between parental plant and offspring family in tetraploid Steel-rye.*

Parent/offspring		No. offspr.		Mean square		r	t^2	P
		plants	fam.	Parent	Offspr.			
Pop/S_1	46/47	199	65	50.7	26.7	0.380	10.598	0.01—0.001
	47/48	441	136	41.1	15.8	0.385	23.277	<0.001
	48/49	450	218	10.3	30.9	0.136	4.094	0.05—0.01
S_1/S_2	46/47	74	38	23.9	32.8	0.352	5.096	0.05—0.01
	47/48	408	108	96.2	27.1	0.534	42.252	<0.001
	48/49	424	216	47.0	45.8	0.601	120.898	<0.001
S_2/S_3	47/48	186	35	84.3	8.2	0.180	1.135	>0.2
	48/49	312	157	106.1	73.0	0.446	38.508	<0.001

the small class of 50—100 % self set). The combined evidence points to the view that in the tetraploids the variation in self-seeding ability is largely heritable, but that external factors modifying in the plus or minus direction have a stronger effect here than at the diploid level.

The increased sensitivity to environmental factors, acting on the self-seeding ability in the tetraploids, will be seen from Table 27. The eight possible parent/offspring correlation coefficients for self seed setting are all positive but indicate a correlation, clearly weaker than that found at the diploid level. These eight correlations are given in more detail in Table 28, which presents the offspring means for self seed set in parental self-seeding classes represented by at least three offspring families. With increased parental seed setting there is only a gradual increase of offspring seeding, and almost exclusively offspring seeding is below the parental means. Indicated are various degrees of pseudo-self-compatibility. True self-compatibility, such as demonstrated at the diploid level, is evidently very rare.

In 1950, when self-seeding was highly promoted, corresponding data show a marked discontinuity in the increase of offspring self seed set, a sudden increase being obtained, when the parent exceeded the limit of 15—20 % self set (S_1 and S_2), 10—15 % (S_3), and 5—10 % (S_4 and S_5). In view of the previous data from 1947—1949 it is considered that this does not indicate a sudden occurrence of true self-compatibility, but merely a larger variation range for the higher degrees of pseudo-self-compatibility.

TABLE 28. *Relation in seed setting upon selfing between parent and offspring plants in tetraploid Steel-rye.*

Offspring means of seed setting in bold types; corresponding numbers of offspring families in thin types.

Offspring		Parental self seed setting in per cent								
Generation	Year	0	5	10	15	20	25	30	35	40
			38	10	8	4	3			
S_1	1947		**4.7**	**5.6**	**8.3**	**8.8**	**8.3**			
			19	14						
S_2	»		**5.8**	**7.0**						
			93	22	8	5	3	4		
S_1	1948		**3.4**	**4.8**	**5.6**	**6.3**	**8.2**	**9.5**		
			33	28	14	17	6	4	3	
S_2	»		**3.0**	**6.5**	**7.3**	**7.2**	**11.5**	**14.4**	**8.2**	
			18	7	4		3			
S_3	»		**3.8**	**4.6**	**4.5**	—	**6.6**			
			164	40	9	4				
S_1	1949		**3.6**	**3.7**	**7.9**	**9.3**				
			111	57	23	11	6	3	5	
S_2	»		**3.8**	**6.7**	**9.5**	**10.1**	**15.7**	**21.8**	**23.5**	
			50	41	30	9	9	7	3	3
S_3	»		**6.3**	**10.8**	**7.6**	**13.3**	**17.0**	**11.7**	**16.7**	**15.6**
Means			**4.3**	**6.2**	**7.2**	**9.2**	**11.2**	**14.4**	**16.1**	
			102	39	13	7				
S_1	1950		**7.8**	**12.4**	**11.3**	**21.6**				
			66	44	24	10	4	5	4	
S_2	»		**10.5**	**14.9**	**18.0**	**25.8**	**15.3**	**32.5**	**33.5**	
			43	32	22	9	8	9	4	5
S_3	»		**12.2**	**14.7**	**24.0**	**27.2**	**25.4**	**19.9**	**24.2**	**28.3**
			30	38	24	18	17	9	6	
S_4	»		**12.6**	**18.1**	**20.2**	**21.6**	**21.1**	**31.6**	**17.5**	
			18	17	8	7				
S_5	»		**14.8**	**23.1**	**21.7**	**20.1**				

TABLE 29. *Open seed set in tetraploid plants with low self-seeding (0—5 %), when the parent plant displayed low or high self-seeding.* (χ^2_c calculated for S_1 and S_2.)

Offspring		Parent self seed set in %	Offspring open seed set in %						No. of offspring		Difference between »low» and »high»			
generation	years		0	5	10	25	50	100	plants	families	χ^2	d. f.	P	Classes pooled
S_1	1947—49	0—10			3	10	91	444	548	309	6.509	1	0.02—0.01	0—50
		10—100				5	41	144	160	46				
Total					3	15	132	558	708	355				
S_2	1947—49	0—10	2	—	4		85	199	290	185	1.812	1	0.2—0.1	0—50
		10—100	1	1	1		33	110	146	66				
Total			3	1	5		118	309	436	251				
S_3	1948—49	0—10	6	—	14		39	32	91	58	3.697	2	0.2—0.1	0—25
		10—100	1	2	13		53	24	93	37				
Total			7	2	27		92	56	184	95				

TABLE 30 a. *Seed setting in families obtained from highly self-seeding tetraploid plants (above 25 % self set) and containing plants with low self-seeding (0—5 % self set).*

Parent/offspring	No. of offspring families		Seed setting in per cent in offspring plants upon																	
			Self-pollination						Open flowering in plants with											
									self seed set below 5 % (»low»)						self seed set above 5 % (»high»)					
	Totally	with low-seeding plants	0	5	10	25	50	100	0	5	10	25	50	100	0	5	10	25	50	100
Pop/S_1 46/47—47/48	6	6	26	20	26	7	1						6	18				1	11	40
S_1/S_2 47/48—48/49	18	10	26	15	23	11						1	6	17					10	38
S_2/S_3 47/48—48/49	19	10	47	31	23	1						6	22	8				3	28	20
Grand total	43	26	99	66	72	19	1					7	34	43				4	49	98

b. *Difference in open seed set between »low-seeding» and »high-seeding» groups.*

Parent/offspring	χ^2_c	d. f.	P	Classes pooled
Pop/S_1	—	—	0.612	10—50
S_1/S_2	—	—	0.307	»
S_2/S_3	—	—	0.075	»
Grand total	3.675	1	0.1—0.05	10—50

TABLE 31. *Nature and inheritance of low and high self-seeding ability in three successive tetraploid generations (Pop. → S_1 → S_2 and S_1 → S_2 → S_3, respectively).*

d. f. = (1) classes 5—50 pooled
d. f. = 2 » 10—50 »

Grandparent		Offspring family self set in per cent when parent self set was												Difference		
		less than 10 %							more than 10 %							
Generation	self set in %	0	5	10	25	50	100	No. offspring families	0	5	10	25	50	100	No. offspring families	χ^2	d. f.	P
Pop	0—10	95	11	5	1			112	11	8	7	2			28	—	(1)	< 0.001
	> 10	71	23	16				110	24	17	23	3			67	17.093	2	< 0.001
Difference		$\chi^2 = 12.229$ d. f. = 2 P = 0.01—0.001							$\chi^2 = 0.383$ d. f. = 2 P = 0.7—0.5									
	0—10	41	9	4				54	11	7	4				22	—	(1)	0.028
	> 10	33	8	16	5			62	14	12	23	5			54	8.973	2	0.02—0.01
Difference		$\chi^2 = 11.995$ d. f. = 2 P = 0.01—0.001							$\chi^2 = 7.530$ d. f. = 2 P = 0.05—0.02									

3. Higher degrees of self-seeding ability

The nature of the difference between high and low self-seeding will now be studied in more detail. Table 29 gives a comparison of general fertility between low-seeding offspring plants, obtained from the low- or the high-seeding parental categories, respectively. The tendency is not clear-cut. In the S_1 offspring, the »low» parentage group has better open seed set; in the S_2 and S_3 offspring no clear differences can be demonstrated between the »low» and the »high» parentage groups. There is no indication that the origin of low-seeding offspring plants may be different for the high-seeding and low-seeding parent groups.

Equally in the offspring from the highest self-seeding parent group (above 25 % self seed set), there is no clear difference in general seeding ability between low- and high-seeding sib plants (Table 30). In view of the inbreeding practiced, the tendency to inferior open seed set that should be expected for the low-seeding category may be considered weak. Evidently, low self-seeding in the present material is mainly due to other factors than disturbances resulting from inbreeding.

Thus, there is no indication that the difference between high and low self-seeding may be anything than a difference in degree of pseudo-self-compatibility. The different degrees of pseudo-self-compatibility are heritable but highly modifiable, as will be evident from Table 31, which gives self set relations in three successive generations (Pop—S_1—S_2 and S_1—S_2—S_3, respectively). High-seeding parents on an average have offspring with higher self set than have low-seeding parents. High-seeding parents derived from low-seeding grandparents on an average have offspring with lower self set than in cases of high-seeding grandparents. Low-seeding parents derived from high-seeding grandparents on an average have offspring with higher self set than in cases of low-seeding grandparents.

The pattern of self-seeding relations is definitely different from that obtained at the diploid level. In view of the apparently larger variation range of pseudo-self-compatibility in the tetraploids, cases of true self-compatibility may be difficult to recognize, but they are evidently much rarer than in the diploids. An attempt to distinguish between supposed high degrees of pseudo-self-compatibility and true self-compatibility is made in Table 32.

High-seeding S_1 plants obtained from population parents with less than 5 % self set, were tested for seeding ability in the S_2 generation. Out of 131 such S_1 families obtained in 1947—1948, nine contained high-

TABLE 32. *Inheritance in the S_2 generation of high self-seeding ability (10 % self set), displayed by tetraploid S_1 plants derived in 1947—1948 from population plants with less than 5 % seed setting upon selfing.*

Case No.	Pop. plant No.	S_1 plant			S_2 family								
					seed set in per cent								
		self set %	open set %	self/open set × 100	self-pollination						open poll.		
					0	5	10	25	50	100	25	50	100
1	47/113	33.7	48.4	69.8			1	—	2	1			4
2	47/147	33.0	56.8	58.1					5				2
3	46/11	23.1	58.2	39.7	4		3	3	1			3	8
4	46/112	19.7	71.1	27.7	3		3	1				1	6
5	46/11	19.6	55.6	35.3	2		—	5	2	1			10
6	47/69	19.1	39.3	48.6				1					1
7	46/112	16.0	77.1	20.8			1	1					2
8	46/5	15.9	56.3	28.2	5		—	1				1	2
9	46/90	13.0	69.8	18.6	3							2	1
10	46/52	12.8	37.0	34.6	7		1	1				2	6
11	47/166	12.1	55.9	21.6	1		—	1					2

seeding plants. The eleven S_1 plants in question are ranged in Table 32 according to self set percentage and were tested in the S_2 generation. Considering the S_2 self set data in relation to general seeding ability, three of the S_1 plants (Nos. 1, 2, and 5 in the table) may be regarded as cases of mutation to self-compatibility. In the absence of a strict genetic analysis of these plants, such as performed at the diploid level, the distinguishment is only made tentatively.

TABLE 33. *Frequency of supposed mutant self-compatible plants in the offspring from tetraploid population plants with less than 5 % self seed set.*

	Pop/S_1	
	1946/1947	1947/1948
a. $\frac{\text{No. self kernels}}{\text{No. selfed florets}}$ in Pop	419/39336	280/30240
b. $\frac{\text{No. mutant plants}}{\text{No. plants, totally}}$ in S_1	1/61	2/147
a × b. Rate of mutant S_1 plants	0.000175	0.000126

The frequencies in which such supposed mutant self-compatible plants were obtained after selfing of nearly 70000 florets of 382 clearly self-incompatible plants from the outbred tetraploid populations of 1946—1947, are given in Table 33. Mutant self-compatibles were obtained at rates of 1.26 and 1.75 per ten thousand selfed florets. From the same considerations as made for the diploids in a previous section, the rate of self-compatible mutant pollen grains would, thus, appear to lie somewhere between 10^{-6}—10^{-8}. In the tetraploids, such pollen would, thus, obtain at a rate about one tenth of that computed for the corresponding diploids.

VI. DISCUSSION

Self-seeding ability, as measured by the amount of seed set within isolation bags, is a compound phenomenon, and the reader is referred to the excellent review of NILSSON (1934) for a thorough discussion of the subject. It is evident that self-seeding ability should preferably be studied under natural conditions after isolation by distance, and that bagging *per se* may cause a reduction of the amount of seed set (HERIBERT NILSSON, 1916). Self seed set data from bagged inflorescences might rather express the ability to set seed under bag, but the influence on seed setting exerted by the bag in the present material is considered relatively small, judging from the practically full self-seeding under bag in the genetically self-compatible material. NILSSON (1934), employing the same type of bag, reached a similar conclusion for the grass species studied by him. It is also evident that, although the studies on self-seeding were restricted to the first three generations of inbreeding, the amount of inbreeding in the materials is considerable, and an increase of the frequency of developmental disturbances and of the sensitivity to external agencies might blur the action of factors directly involved in the self-seeding.

The heritable nature of the self-seeding ability, largely independent of general seeding ability, is obvious in the present material of diploid Steel-rye. From the restricted material studied in clone 21 (only two S and two Z incompatibility factors being represented), it is evident that at least part of the different degrees of self-seeding ability may reflect differences between these incompatibility factors, the het het category being more frequently involved in pseudo-compatible pollinations than the corresponding ho het category. A considerable or at least moderate

number of alleles at both the S and Z loci were presumed for the strain of Steel-rye in a previous paper (LUNDQVIST, 1956), fifteen analysed plants from an outbred population all apparently being heterozygous at both loci, although homozygosity at the individual locus might obtain naturally in the population. The number would appear to be large enough to account alone for the different degrees of pseudo-compatibility observed in the strain. There were, however, indications of genes other than S and Z, modifying the self-seeding ability. It should be expected that their action is difficult to distinguish in the balanced polygenic system within the diploid cross-fertilized population (MATHER, 1943). Even in the inbred populations the range of variation for the different degrees of self-seeding ability is rather narrow. It is not possible here to settle which parts of the increased variation in the inbred material may be directly due to this balanced system being upset, or to an increased sensitivity to external disturbances.

In the tetraploid strain of Steel-rye, self-incompatibility has remained quite pronounced in several years' investigations. The persistence of self-incompatibility at the tetraploid level in rye is probably of common occurrence and has been demonstrated by BRESLAWETZ (1940) in a restricted material of spring rye, PLARRE (1954) in Petkus winter rye, and by LEWIS (1954). As was found for the diploids, general seeding ability seems to be only a secondary factor affecting self-seeding ability in the tetraploid outbred populations; but on an average the tetraploids set more seed upon selfing than do the diploids, and the variability in self seeding between years and in parent/offspring relations is highly increased. In a previous discussion of the degrees to which homozygous detrimentals and aneuploidy might be able to affect self-seeding in rye at the diploid and tetraploid levels, it was concluded that fertilization after selfing really occurs more easily in the tetraploids and that, probably, the observed weakening of self-incompatibility is an intrinsic property of polyploidy in rye (LUNDQVIST, 1957). The increased variability in self seed set relations may be explained from changed interaction conditions in the diploid pollen for the incompatibility factors themselves (LUNDQVIST, 1957), or from the polygenic balance becoming upset after doubling of the chromosome number.

In the diploid populations, indications were obtained that there might not always be a difference in degree between low self-seeding and high (above 10 % seed set). It was concluded that high- and full-seeding plants in the self offspring from low-seeding parents were mainly due to mutations directly involving the incompatibility loci S and Z and

eliminating the previously determined pollen specificities. That such high-seeding offspring plants mainly result from the functioning of a mutant pollen grain and not from rare segregation of modifiers is indicated in several ways. (1) There is a tendency that high-seeding S_1 offspring are more frequently obtained from extremely low-seeding than from moderately self-seeding parents. From population plants with self set below 5 %, 372 S_1 plants below, and 42 plants above 25 % self set were obtained, whereas for the parental class of 5—10 % self set the corresponding ratio was 54/2 (see Table 9). The difference corresponds to P=0.081. Similar data were obtained for Vjatka rye (AGEEW, 1929, Table XIX) where the S_1 offspring ratio for population parent plants below 10 % self set was 38/9, and for parents above 10 % self set, 35/3. Such results might indicate that self-compatible mutant pollen grains have to stand a competition with pseudo-compatible grains. (2) Practically no low-seeding offspring plants were obtained, when seven selected highly self-seeding inbred lines were crossed to low-seeding population plants, the aberrant plants evidently being due to other causes than incompatibility. (3) A genetical analysis demonstrated a direct connection between a mutant factor and one of the incompatibility Z alleles, the result being an elimination of the pollen specificity previously determined by that factor.

The occurrence of such mutant self-compatible plants is indicated elsewhere in the literature on rye self-seeding (HERIBERT NILSSON, 1916, 1953; BREWBAKER, 1926; AGEEW, 1929; PETERSON, 1934). PETERSON (1934) and HERIBERT NILSSON (1953) made the cross high-seeding × low-seeding line, but without knowledge of the incompatibility system at work no adequate interpretation was possible. Remaining heterozygosity for incompatibility factors in the high-seeding lines studied by PETERSON evidently complicated his results. HERIBERT NILSSON found a majority of high-seeding plants in his offspring generations, the ratio of plants below and above 10 % self set being 1 : 20 in the pooled material, and he postulated a segregation effected by a minimum of two polymeric factors for self-seeding.

It is important that in the present material a high proportion of low-seeding self offspring from high-seeding plants were shown to be due to inbreeding depression. It is also very suggestive that, when inbreeding could be avoided (high-seeding inbred line crossed to low-seeding population plant), practically no low-seeding plants were obtained. HERIBERT NILSSON (1953) equally obtained no low-seeding plant among the ten F_1 individuals from his cross low × high. MÜNTZING (1943) in a

material of double crosses between self-compatible inbred lines found no low-seeding plants, and in fact the self set exceeded the open set. It appears, although pertinent data are lacking, that many of the irregularities reported in the literature, when high-seeding plants produce strains of low average fertility, and *vice versa* (HERIBERT NILSSON, 1916; BREWBAKER, 1926; AGEEW, 1929; PETERSON, 1934), might have been due to the inbreeding practiced. It is obvious that in the way in which self-compatibility is inherited this character may be easily introduced into any self-incompatible material. This may be of high importance in an inbreeding program.

It is of importance in this connection that no deleterious effect on viability could be demonstrated to result from the mutant, self-incompatibility factor, neither in the pollen, nor in the diploid plant, even when homozygous. The material is small admittedly. LEWIS (1949 b) equally found no clear effect on viability and fertility of mutant S factors induced by X-rays in *Oenothera organensis*. Indications of a reduction of the functioning ability of self-compatible pollen from a spontaneous self-compatible plant in *Trifolium repens* were found by ATWOOD (1945). PANDEY (1956) suggests that permanent loss of the incompatibility reaction in *Trifolium pratense* may be lethal. In rye, FERWERDA (1951) found that self-compatible inbred lines (from MÜNTZING's material) emasculated and dusted with a mixture of equal parts of own and foreign pollen, all yielded a progeny, almost entirely consisting of hybrid individuals. The source of the pollen was not mentioned. It should be expected that in a mixture of foreign pollen the probability will be high of finding pollen superior to the own pollen where practically only a single genotype may be represented.

Nevertheless, there may be cases of true incompatibility in offspring derived from a self-compatible parent. One possible explanation would be that a factor linked to one of the incompatibility loci might account for self-compatibility by means of a pre-meiotic inhibition of the pollen action of the linked incompatibility factor. Cross-over pollen, carrying the previously linked factor in question, would then be compatible in the self style, and self-incompatible offspring may result. Especially in the light of the two self-compatibility loci, demonstrated to be at work in the seven inbred lines of rye, this seems, however, a less probable complication. On the other hand, self-incompatible plants may result from the functioning of pseudo-compatible pollen in plants heterozygous for a mutant self-compatibility factor.

It was mentioned above that, obviously, pseudo-compatible pollen

could successfully compete with self-compatible mutant pollen grains. This indicates that the growth rate of the pseudo-compatible pollen may equal that of self-compatible and foreign pollen. In view of the low growth rate of pollen in the self style, observed in rye (*e. g.*, SEARS, 1937), the question arises whether pseudo-compatible grains in rye might be due to a temporary loss of the pollen activity of an incompatibility factor. Such »revertible» mutations have been inferred for *Oenothera organensis* (LEWIS, 1951; LEWIS and CROWE, 1953), primarily from an increase in frequency after X-raying; after fertilization the mutant factor reverts to its previous activity, and the resultant plant is self-incompatible. In rye, cases of high-seeding plants with exclusively or almost exclusively low-seeding offspring may be due to the inbreeding practiced or to experimental errors; but they may also indicate the functioning of a cluster of revertible mutant pollen.

It is a striking fact that, whereas the number of mutable factors is doubled upon chromosome doubling, yet the frequency of self-incompatible plants giving rise to self-compatible mutants in the offspring after selfing is much lower in the tetraploids. Clearly, part of the reduced frequency of self-compatible mutants might be due to the heavier competition with pseudo-compatible pollen which the mutant self-compatible pollen has to stand in the more pseudo-compatible tetraploid strain. The main reason is, however, that a single mutation will not make the self diploid pollen react compatibly.

Combined evidence at the tetraploid level, reviewed and discussed in detail in a previous paper (LUNDQVIST, 1957), pointed to the view that the pollen specificities result from co-operation between the loci within pairs of factors. One such factorial pair of the pollen matched in the style is sufficient for incompatibility. The haploid pollen carries only one such pair of co-operating factors; for the four incompatibility factors of the diploid pollen several ways of such co-operation may be possible. Accordingly, an incompatibility factor mutation which carried in the haploid pollen would cause it react compatibly in the self style, will behave in a »recessive» way in the diploid pollen. Evidently both loci are capable of such mutations that will render the self haploid pollen compatible, but in the diploid pollen such mutations will not be recovered unless by means of double reduction two mutant alleles happen to occur in the same grain. Thus, it is evident that, at the diploid level, the rate of self-compatible mutant pollen will be equal to the mutation rate; but not at the tetraploid level.

Considering the gametophytic determination of the pollen specificities,

the persistence of self-incompatibility at the tetraploid level in rye is remarkable. Tendencies to a breakdown of the incompatibility mechanism are common in species with gametophytically controlled self-compatibility (for a review of the literature, see ATWOOD and BREWBAKER, 1953; BREWBAKER, 1955, gives a list of »gametophytic» species). It appears that in these species there has been no opportunity for selection for the ability of the incompatibility genes to work efficiently in pairs on the male side (LEWIS, 1947; ATWOOD and BREWBAKER, 1953). In species where sporophytic control of the pollen specificities has been established or can be inferred, incompatibilities are, in any case, much less affected by chromosomal doubling (see lists of species given by ATWOOD and BREWBAKER, 1953; and BREWBAKER, 1955).

It is important at this stage to note that there is direct genetical evidence that *both* the S and Z loci are active post-meiotically, at the *gametophyte* stage, and not in the way that, *e. g.*, one locus is active pre-meiotically (sporophytically), the other locus post-meiotically (gametophytically) (LUNDQVIST, 1956). New evidence pointing in the same direction may be obtained from the self-compatible inbred lines of rye reported above. Except line 19, these lines were probably homozygous at either S or Z for self-compatibility factors. From the pollination by self-incompatible population plants, the resultant F_1 plants would then be either $S_{f.a}\,Z_{c.d}$ or $S_{a.b}\,Z_{f.d}$, the *f* subscript denoting a self-compatibility factor, the other subscripts, ordinary incompatibility factors. Assuming the S locus to be active at the sporophytic stage, the self pollen of a plant $S_{f.\bar{a}}\,Z_{c.d}$ might be expected to react incompatibly, as it has been demonstrated that one incompatibility factor pair of the pollen matched in the style is sufficient for incompatibility (LUNDQVIST, 1957). Without exception, however, all F_1 offspring were self-compatible.

The occurrence of two incompatibility factors in the haploid pollen of rye may have a direct bearing on the persistence of self-incompatibilities at the tetraploid level. The two-loci gametophytic system has been established in the two other grass species where so far a strict genetical analysis of incompatibilities has been performed, *viz.*, *Festuca pratensis* (LUNDQVIST, 1955 and unpublished) and *Phalaris coerulescens* (HAYMAN, 1956). Both of them are diploid with $2n=14$; but the incompatibilities at the tetraploid level have not yet been studied in these species. Assuming a wide distribution in the grass family of the rye system, it might, however, be expected that polyploid grasses with pronounced self-incompatibility should be common. The ratios open/self seed set (F/S) have been gathered from the literature for a number of

TABLE 34. *Ratio open/self seed set (F/S) in grass species*

F data obtained from open flowering. S data obtained from isolation in

	Chromosome number	Isol. method	Ratio F/S	Author
Diploid species				
Alopecurus myosuroides	14	2	36.50	BEDDOWS, 1931
Apera spica venti	14	2	35.30	» »
Beckmannia erucaeformis	14	3	14.87	TROLL, 1931
Briza media	14	2	2168.30	BEDDOWS, 1931
Cynosurus cristatus	14	3	5.16	TROLL, 1931
» »	»	2	13.86	BEDDOWS, 1931
Elymus junceus	14	1	296.50	SMITH, 1944
Festuca pratensis	14	1	5.03	» »
» »	»	3	5.88	TROLL, 1931
» »	»	1	8.80	HAYES and SCHMID, 1943
» »	»	3	22.42	NILSSON, 1934
Holcus lanatus	14	2	139.71	BEDDOWS, 1931
Hordeum bulbosum	14	1	∞	LEIN, 1948
Lolium multiflorum var. *westerwoldicum*	14	3	5.33	TROLL, 1930, 1931
Lolium multiflorum	»	3	16.98	» » »
» »	»	3	31.81	NILSSON, 1930
» » var. *westerwoldicum*	»	2	101.73	BEDDOWS, 1931
Lolium perenne	14	3	3.87	TROLL, 1930, 1931
» »	»	3	4.68	NILSSON, 1933
Phalaris canariensis	12	2	4.82	BEDDOWS, 1931
Phleum phleoides	14	1	425.00	SMITH, 1944
Polyploid species				
Agropyron cristatum	(14), 28	1	11.97	MURPHY, 1942
» »	»	1	46.17	SMITH, 1944
» *intermedium*	42	1	33.43	» »
» *repens*	28, 42	1(?)	9.09	BEDDOWS, 1931
» »	»	1	49.60	SMITH, 1944
» *sibiricum*	28	1	551.00	» »
» *smithii*	28, 42. 56	1	45.70	» »
» *trichophorum*	42	1	18.92	» »

where self-incompatibility has been established or inferred.

(1) parchment bags, (2) transparent glazed paper bags, (3) pergamine bags.

	Chromosome number	Isol. method	Ratio F/S	Author
Agrostis stolonifera	28	3	34.46	SPLECHTNER, 1925
Alopecurus pratensis	28	3	2.96	TROLL, 1930, 1931
Arrhenaterum elatius	28	3	5.15	» » »
» »	»	1	21.00	SMITH, 1944
Bromus erectus	56	1	7.12	BEDDOWS, 1931
» »	»	1	10.76	SMITH, 1944
» *inermis*	56	3	5.73	TROLL, 1931
» »	»	1	8.47	WILSIE *et al.*, 1952
» »	»	1	13.39	SMITH, 1944
» »	»	1	59.25	HAYES and SCHMID, 1943
» »	»	1	79.12	BEDDOWS, 1931
Dactylis glomerata	28	3	4.04	SYLVÉN, 1929 b
» »	»	1	4.90	MYERS, 1942
» »	»	1	6.17	SCHULTZ, 1941
» »	»	3	9.87	TROLL, 1930, 1931
» »	»	1	10.97	SMITH, 1944
» »	»	1	25.90	HAYES and SCHMID, 1943
» »	»	1	27.14	FRANDSEN, 1917
Deschampsia flexuosa	28	2	18.30	BEDDOWS, 1931
Elymus giganteus	28, 56	1	46.20	SMITH, 1944
» *triticoides*	28	1	128.00	» »
Festuca arundinacea	28. 42, 70	1	3.83	» »
» »	»	3	7.09	TROLL, 1930, 1931
Hordeum bulbosum	(14), 28	1	16.84	SMITH, 1944
» »	28	1	24.33	LEIN, 1948
» »	(14), 28	1(?)	∞	BEDDOWS, 1931
Oryzopsis hymenoides	48	1	2.92	SMITH, 1944
Phalaris arundinacea	28	3	10.24	TROLL, 1930, 1931
» »	»	1	23.83	SMITH, 1944
Phleum pratense	42	3	6.39	SYLVÉN, 1929 b
» »	»	3	9.15	TROLL, 1931
» »	»	3	11.24	WITTE, 1922
» »	»	3	37.51	VALLE, 1931,
» »	»	3	46.48	» »
» »	»	1	91.30	FRANDSEN, 1917

TABLE 35. *Ratio cross/self seed set (F/S) in species where a one-locus*

Both F and S data almost exclusively

Families and species	Chromosome number	Ratio F/S	Author
Leguminosae			
Trifolium hybridum	16	25.52	MOORE, 1917
» »	»	128.83	JØRGENSEN, 1921
» »	»	238.10	WILLIAMS, 1951
» *nigrescens*	16	∞	BREWBAKER, 1955
» *pratense*	14	51.25	RINKE and JOHNSON, 1941
» »	»	57.22	SYLVÉN, 1929 a
» »	»	72.05	WILLIAMS, 1931 a
» »	»	98.65	WILLIAMS and WILLIAMS, 1947
» »	»	110.84	WEXELSEN, 1945
» »	»	114.25	FRANDSEN, 1917
» »	»	115.46	WEXELSEN, 1945
» »	»	736.62	SCHLECHT, 1922
» *repens*	32	26.71	JØRGENSEN, 1921
» »	»	31.39	WILLIAMS, 1931 b
» »	»	31.98	ATWOOD, 1941
» »	»	48.78	NILSSON-LEISSNER, 1934
» »	»	56.02	» »
Onagraceae			
Oenothera organensis	14	6722.62	LEWIS, 1948, 1949 b
Papaveraceae			
Papaver rhoeas	14	17.16	MOORE, 1917
Rosaceae			
Prunus amygdalus	16	81.00	TUFTS and PHILP, 1922

self-incompatible diploid and polyploid grasses (Table 34). The chromosome numbers are mainly according to LÖVE and LÖVE (1948) and DARLINGTON and WYLIE (1955). No complete review of the literature has been attempted here, but the common occurrence of polyploid grasses with clear self-incompatibility is quite obvious. Different degrees of self-incompatibility seem to be represented both within and between species, but there is a slight tendency to a more accentuated self-incompatibility in the diploids, species with an F/S ratio above 50 being more common here ($P=0.132$).

Corresponding data for species where an incompatibility system with

gametophytic incompatibility system has been established or inferred.

obtained from artificial pollination.

Families and species	Chromosome number	Ratio F/S	Author
Prunus avium	16	28.64	JOHANSSON and CALLMAR, 1936
» »	»	376.33	CRANE and BROWN, 1937
» »	»	∞	JOHANSSON, 1945
» *cerasus* (and »Duke Cherries»)	32	2.01	CRANE, 1927
» *domestica*	48	2.75	JOHANSSON, 1945
» »	»	2.81	» 1956
» »	»	3.29	CRANE and LAWRENCE, 1931
» »	»	4.49	JOHANSSON and CALLMAR, 1936
Pyrus communis	34	7.06	JOHANSSON, 1945
» »	»	7.90	TUFTS and PHILP, 1923
» »	»	10.80	JOHANSSON, 1956
» »	»	18.67	CRANE and LEWIS, 1942
» »	»	106.67	» » » »
» *malus*	34	18.14	JOHANSSON, 1945
» »	»	106.00	» 1956
Scrophulariaceae			
Veronica syriaca	14	> 100(?)	LEHMANN, 1919
Solanaceae			
Lycopersicon peruvianum	24	570.00	MCGUIRE and RICK, 1954
Nicotiana alata	18	13.33	EAST and PARK, 1917
» *forgetiana*	18	150.00	» » » »
Petunia axillaris	14	∞	STOUT, 1952
» *integrifolia*	14	∞	» »
Solanum caldasii	24	∞	PUSHKARNATH, 1942
» »	»	∞	» 1945

one gametophytic locus has been established or can be inferred, are gathered in Table 35. It may be seen that for the diploid species the F/S ratios are generally higher than in the grasses. Now, such data may be difficult to compare, because the grasses have a one-seeded ovary, whereas most of the species in Table 35, except those belonging to the genus *Prunus*, have many-seeded ovaries which may need a minimum of fertilized ovules in order to develop and avoid abscission. Besides, the species in Table 35 are insect pollinated, but the grasses are wind pollinated, and there are indications that the selective value of the self-seeding ability may depend on the population structure. In Russian rye

ANTROPOV and ANTROPOV (1929) found an increase of self-seeding plants from the south to the north, and wild ryes were more self-sterile than cultivated ryes. KRANZ (1957) on the contrary, found that primitive ryes from Iran, when cultivated in Germany, were more self-fertile than the commercial Petkus rye. DUCKART (1928) found a higher degree of self seed set in an indigenous rye in comparison to highbred German varieties. It is interesting that a high degree of similarity in the pattern of varietal self seed set between different years has been observed (DUCKART, 1928; SURIKOV, 1956 a; the present data from Steel-rye).

It is striking, however, that in Table 35 the polyploid species are rare and clearly less self-incompatible. The considerably higher frequency of self-incompatibility in polyploid grasses is also evident from the reviews of EAST (1940) and FRYXELL (1957). While part of this difference may be due the higher frequency of polyploids in the grasses, it is considered that there is indicated a fundamental difference between the one-locus and the two-loci gametophytic incompatibility systems.

For an evaluation of this difference, the following characteristics of the two-loci system in rye may be important. (1) The loci are most probably independently inherited and multiallelic. (2) They are both post-meiotically active in determining the pollen specificities. (3) They are both capable of mutations rendering the haploid pollen compatible in the self style. (4) One of the loci has given evidence of a compound structure, the mutation eliminating the pollen specificity previously determined by the locus, but leaving the style action unaffected. (5) The loci act co-operatively in the elaboration of the pollen specificities. (6) This co-operation within pairs of incompatibility factors is still efficient, when more than two factors are present in the pollen.

It is obvious that the rye incompatibility loci have several traits in common with the S locus of the one-locus gametophytic system: time of action, multiallelism, and mutability. Investigations are designed to see whether both loci give evidence of a compound structure with pollen- and stylar-active parts. Such evidence has been advanced from *Oenothera*, *Petunia*, *Prunus*, and *Trifolium* (LEWIS, 1949 b; STOUT, 1952; PANDEY, 1956). It is also obvious that no definite difference between the two loci could be established. So far, a duplicative origin of the two-loci system does not appear improbable.

At this point, it is essential that the two loci in the rye system act co-operatively, and that they still co-operate in an efficient way after chromosomal doubling. The co-operative elaboration of the incompati-

bility specificities appears to be successful in practically any combination of an S and a Z allele, judging from the predominance of self-incompatible plants in the outbred populations of Steel-rye. This successful co-operation between two multiallelic loci seems to imply only small differences between the alleles within each of these loci. Probably, the primary allelic differences do not need to be great to be able to ensure the unique specificity of the end products in a co-operative system. Thus far, after chromosomal doubling, unbalanced interaction between two different alleles, which may now occur together at both S and Z in the diploid pollen, would be less probable, and the persistence of self-incompatibility at the tetraploid level might find an explanation.

The nature of the allelic interaction in heterogenic diploid pollen of species with a one-locus gametophytic system is not precisely known (LEWIS, 1943, 1947, 1952). The functioning of such pollen in the self style has been explained to result from competition between the alleles, reducing the amount of the allelic products. Alternatively, the picture of the two alleles interacting to produce a product with a new specificity was suggested. It appears that the latter explanation has become more probable after the present observations in the grass incompatibilities. Such co-operative interaction between different loci in the case of incompatibility factors has been observed also in the tetrapolar fungus *Schizophyllum commune* (PAPAZIAN, 1951). Whether there are such new, co-operatively elaborated substances, and whether they might be capable of taking over the incompatibility control, previously exerted by a single locus, are questions which might find an answer from a continued study of grass incompatibilities.

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SUMMARY

(1) The amounts of seed set upon selfing or after genetically incompatible pollinations at the diploid and tetraploid levels have been studied in the commercial variety of Steel-rye. In neither strain is there absolute self-incompatibility, and self seed is frequently obtained in varying amounts.

(2) In 19 diploid families derived in various ways of inbreeding from a single plant, self seed set and frequency of self-seeding plants show a decrease with increasing amount of inbreeding. Comparing het het and ho het plants, frequency and degree of pseudo-compatibility are clearly higher in the het het group. There is a tendency within both groups that pseudo-cross-compatibility is higher for the pseudo-self-compatible category, both at the female and the male side. Indicated are heritable degrees of pseudo-compatibility due to the incompatibility loci S and Z, but also determined by other factors.

(3) In diploid outbred populations, about 95 % of the plants are found below the limit of 5 % self set, and less than one per cent give a reasonably full seed set upon selfing.

(4) In the inbred derivatives of these populations, high-seeding offspring may be obtained from a low-seeding parent, and *vice versa*, but heritable degrees of self-seeding ability are clearly demonstrated. Self seed set variation due to chance does occur, but the range of this variation seems to be rather narrow. Opposed to the depression effect of inbreeding, resulting in lowered seed setting, is the occurrence of offspring plants with relatively increased self-seeding ability. A fundamental difference between low- and high-seeding plants is indicated.

(5) Self-compatibility resulting from a mutation at the Z locus, eliminating the pollen specificity previously determined by it, but leaving the style action unaffected, indicates a compound structure of this locus. As far as can be seen, the mutant factor does not reduce the functioning ability of pollen carrying it, nor does it impair the viability of the self-compatible plants.

(6) Self-compatibility in seven diploid practically homozygous inbred lines is shown to be due to two independently segregating loci with self-compatibility factors. The pollen grows down the self style, when it carries at least one such factor at either locus. The mutant factor was four times associated with one of the loci, four times with the other. Direct proof is lacking, but it is concluded that these two loci are identical to the incompatibility loci S and Z.

(7) Indications are obtained that also in the diploid populations studied, high and full self-seeding are predominantly effected by mutant factors acting in the above way. The rate of self-compatible mutant pollen grains is computed to lie somewhere between 10^{-5}—10^{-7}.

(8) In tetraploid outbred populations about 80—85 % of the plants are found below the limit of 5 % self set. Polyploidy has introduced a

clear weakening of self-incompatibility, but fully self-seeding plants are not obtained.

(9) In the inbred derivatives of the tetraploid populations there is a pronounced skewness towards low offspring self-seeding for practically all parental self seed set classes. The combined evidence points to the heritability of the self-seeding ability, but in comparison to the diploids there is an increased variability in self-seeding relations between years and between generations.

(10) High self-seeding due to mutant pollen is extremely rare in the tetraploids. The main reason is that a single mutation will not be sufficient to make the self diploid pollen react compatibly, as one factorial pair of the pollen matched in the style will suffice to make it react incompatibly. Thus, such mutations will not be recovered in the diploid pollen unless by means of double reduction two mutant alleles happen to occur in the same grain. In the diploids, the rate of self-compatible mutant pollen will be equal to the mutation rate; but not in the tetraploids. The rate of self-compatible diploid pollen is computed to about one tenth of that computed for the haploid pollen.

(11) The persistence of self-incompatibility in rye after chromosomal doubling is discussed in relation to species with a one-locus gametophytic incompatibility system. The latter species often display an impairment or breakdown of incompatibilities at the polyploid level. Considering the high frequency of self-incompatible polyploid grass species, it is considered that there is indicated a fundamental difference between the one-locus and the two-loci gametophytic incompatibility systems.

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ABBERRATIONS IN THE NUCLEOLAR CHROMOSOME OF INBRED RYE

III. SIZE VARIATION IN INBRED LINES IN RELATION TO VIGOUR

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I. INTRODUCTION

IN continuation with the previous line of work (BOSE, 1956, 1957) more cases of aberrations involving the nucleolar chromosomes have been found, in several more inbred lines; these have been described in detail in the present paper.

The term aberration has been used here in a broad sense and describes the variations found in lengths of the arms and satellites, of different inbred lines, commonly caused by the addition or deletion of heterochromatic material residing in their knob formation regions.

The question arises if any normal (nucleolar) chromosome type can be said to exist. In view of the fact that so many different combinations of chromosome segments have been found in inbred lines and in population plants, no definite answer can as yet be given. It can be pointed out, however, that certain regular chromosome patterns (*e. g.* combinations of certain types of short arms and satellites together with special types of L.A./S.A. ratios) have been found to occur more frequently than others; and it has often been useful to regard them as standard types.

In previous papers mention was also made, about the possible relationship, which might exist between these types of chromosome aberrations and inbreeding behaviour. An examination of this suggestion has been made in this paper, based on observations made on some aspects of vegetative growth and seed setting ability in a few lines with known chromosome constitution.

II. MATERIAL AND METHODS

The lines examined in the present paper, came from the same stock of inbred material, from which several other lines had already been previously investigated (BOSE, *l. c.*). The methods followed in fixing and staining, and the technique employed in measuring the chromosomes, or parts of the chromosomes, have also been adequately described before. A few details may however, be mentioned here. Root tips were obtained from freshly germinating kernels grown on moist filterpaper. They were immersed in a solution of oxyquinoline (of 0.003 M/L. concentration) for six hours at 16° C and subsequently stained and squashed, using the usual aceto-orcein squash method (TJIO and LEVAN, 1950).

The following nine lines have been examined in this paper. Line Nos. 26, 30, 48, 50, 57, 72, 79, 91 and 108; the numbers of the lines belong to the year 1957. With some lines, however, kernels from plants grown both in the years 1956 and 1957 had to be used. This was because the number of kernels obtained for one year in these lines were too few to be of use.

In the second part of the paper, some details have been given of the methods used in studying differences in the vegetative growth and the flowering and seed setting abilities, of some of the inbred lines.

III. DEFINITIONS

The meaning of most of the terms that have been used here, have already been explained before (BOSE, *l. c.*). The term chromosome pattern however needs special mention. This term (applied only in case of the nucleolar chromosome) refers to the different types of short arm-satellite combinations that are possible. It has been found for example, that chromosomes whose L.A./S.A. ratios are high (*i. e.* 2.3 or more) usually have small short arms and large satellites. (This is equivalent to the aS/aS type of chromosome, reported in earlier papers. This combination of large satellites together with small short arms belongs to a special pattern. Another pattern (or grouping) commonly met with is the As/As type (where capital »A» represents a large short arm and a small »s» a small satellite and vice versa). Changes in the combination of segments may be produced by crossing over, in selfed heteromorphic plants. This is due to the fact that crossing over in rye frequently occurs in the nucleolar organising region (BOSE, 1956).

IV. OBSERVATIONS

1. Mitosis

In Table 1 is given the distribution frequency of the ratios of the nine inbred lines that have been mentioned before. They have been arranged in the table in such a way that lines with high ratios ($\bar{x}=2.20$ or more) come first and then come the medium ratio lines ($\bar{x}=2.1$—2.0) and, at the bottom, all those lines with ratios less than 2.00. In the previous paper (BOSE, 1957) the three groups as formed above have been designated as group A, group B and group C, respectively. There are four lines belonging to group A, and these are lines 108, 72, 57 and possibly 26. Their respective L.A./S.A. ratios are 2.44, 2.34, 2.24 and 2.20. In the second group (B) there is only one line present, line 30, its ratios being 2.10. In the third group (C), there are four lines *viz.* lines 50, 79, 91 and 48 and their respective ratios are 1.96, 1.93, 1.92, and 1.88.

The groupings made here are not so clear (especially in lines belonging to group A) and straight-forward as was the case with lines that had been investigated before (BOSE, *l. c.*). Thus it can be seen from Table 3, where the calculated values of »t» for the ratios of the different lines are given, that there are often significant differences present in lines belonging to the same group. In group A for instance, line 108 differs significantly from line 57 ($t=4.128$, $P<0.001$) as well as from line 26 ($t=5.714$, $P<0.001$). While there were differences between lines, belonging to the same group (A) in the lines investigated before, these differences were never so big. Lines belonging to group C are however more uniform, though even here some difference exist between lines 40 and 58.

In Table 2 detailed measurements are given of the different regions of the nucleolar chromosomes belonging to the lines mentioned above, and the same grouping as in the previous table has been kept here. In Tables 3 to 7 are shown the values of »t» that have been obtained on comparing the ratios and the different regions of the nucleolar chromosomes of the nine inbred lines. A brief summary of their more noteworthy characters is given below. Line 91 (Fig. 6) has the largest chromosome length ($\bar{x}=34.60$) of all the nine lines. This line belongs to group C and has a ratio of 1.92. It does not show any significant difference with respect to this character, when compared with other lines belonging to the same group (*i. e.* lines 57, 79 and 48 — see also Table 4). On the other hand there are significant differences between line 91 (and other lines belonging to this group) and nearly all the lines

TABLE 1. *Distribution frequency of the L.A./S.A. ratios of the nine inbred lines investigated in the present paper.*

Group	Line no. (1957)	Ratio:	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	N	$\bar{x}$	m
A	108								2	2	1	6	11	1	5	1	1	30	2.44	0.035
	72								3	6	1	6	11	1	1	1		30	2.34	0.032
	57								4	10	6	7	2	0	0	1		30	2.24	0.028
	26						1	0	5	11	7	3	3	0				30	2.20	0.024
B	30							3	14	10	0	3						30	2.10	0.020
C	50					3	7	8	10	0	2							30	1.96	0.022
	79					2	11	9	7	1								30	1.93	0.017
	91				3	3	9	5	5	5								30	1.92	0.028
	48			1	1	4	11	10	2	0	1							30	1.88	0.024
Total for the 9 lines			1	4	12	39	35	52	45	18	25	27	2	6	3	1		270		

TABLE 2. *Summary of the lengths of the different regions of the nucleolar chromosome in the inbred lines described in the text.*

Line No.	Ratio L. A./S. A.			Tot. chrom. length			Long arm length			Short arm length			Satellite length			Group
	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	
108	2.44	0.035	30	30.87	0.466	30	18.73	0.290	30	8.20	0.174	30	5.13	0.101	30	A
72	2.34	0.032	30	30.47	0.408	30	17.93	0.248	30	8.00	0.133	30	5.57	0.082	30	
57	2.24	0.028	30	30.07	0.657	30	17.40	0.419	30	8.20	0.198	30	5.47	0.101	30	
26	2.20	0.024	30	33.30	0.662	30	19.33	0.364	30	9.27	0.202	30	5.67	0.097	30	
30	2.10	0.020	30	33.60	0.560	30	19.00	0.321	30	9.53	0.182	30	6.07	0.114	30	B
50	1.96	0.022	30	34.17	0.653	30	19.80	0.393	30	10.67	0.230	30	4.70	0.088	30	C
79	1.93	0.017	30	33.57	0.484	30	18.77	0.389	30	10.27	0.218	30	5.57	0.066	30	
91	1.92	0.028	30	34.60	0.506	30	18.97	0.294	30	10.40	0.175	30	6.23	0.117	30	
48	1.88	0.024	30	34.30	0.566	30	18.83	0.326	30	10.63	0.213	30	5.83	0.111	30	

All measurements are given in millimetres at a magnification of ca 3500×.

TABLE 3. *Values of »t» obtained on comparing the L.A./S.A. ratios of the nine inbred lines described in Table 1.*

No. of chrom. obs.	30	30	30	30	30	30	30	30		
Line No.	72	57	26	30	50	79	91	48		
	2.000	4.128	5.714	8.350	11.634	13.000	11.489	13.262	108	30
		2.381	3.650	6.486	9.821	11.472	10.071	11.575	72	30
			1.243	4.000	7.861	9.485	8.075	9.811	57	30
				2.938	7.182	8.900	7.486	9.057	26	30
					4.767	6.654	5.229	6.969	30	30
						1.071	1.111	2.424	50	30
							0.303	1.667	79	30
								1.081	91	30

In this and the following tables (Table Nos. 4, 5, 6 and 7) values of »t» above 3.551 indicate highly significant differences ($P < 0.001$). Values of »t» between 3.551 and 2.704 indicate significant differences ($0.001 < P < 0.01$). Values of »t» between 2.423 and 2.021 are probably significant ($0.01 < P < 0.05$). Values of »t» below 2.021 are considered to be non-significant.

TABLE 4. *Values of »t» obtained on comparing the total chromosome lengths of the nine inbred lines. (From data given in Table 2.)*

No. of chrom. obs.	30	30	30	30	30	30	30	30		
Line No.	72	57	26	30	50	79	91	48		
	0.646	0.994	3.004	3.749	4.212	4.018	5.426	4.683	108	30
		0.517	3.641	4.521	4.805	4.897	6.358	5.491	72	30
			3.465	4.094	4.428	4.289	5.468	4.882	57	30
				0.346	0.932	0.326	1.563	1.148	26	30
					0.659	0.045	1.325	0.879	30	30
						0.738	0.524	0.154	50	30
							1.476	0.984	79	30
								0.395	91	30

TABLE 5. *Values of »t» obtained on comparing the long arm lengths of the nine inbred lines. (From data given in Table 2.)*

No. of chrom. obs.	30	30	30	30	30	30	30	30		
Line No.	72	57	26	30	50	79	91	48		
	2.094	2.614	1.288	0.617	2.186	0.070	0.564	0.229	108	30
		1.094	3.182	2.628	4.015	1.809	2.686	2.195	72	30
			3.483	3.030	4.181	2.390	3.061	2.699	57	30
				0.687	0.871	1.082	0.782	1.022	26	30
					1.578	0.462	0.076	0.365	30	30
						1.868	1.697	1.892	50	30
							0.410	0.130	79	30
								0.305	91	30

TABLE 6. *Values of »t» obtained on comparing the short arm (minus the satellite region) lengths of the nine inbred lines. (From data given in Table 2.)*

No. of chrom. obs.	30	30	30	30	30	30	30	30		
Line No.	72	57	26	30	50	79	91	48		
	0.913	0.000	3.996	5.290	8.566	7.409	8.907	8.847	108	30
		0.837	5.236	6.813	10.026	8.890	10.909	10.490	72	30
			3.770	4.955	8.142	7.031	8.333	8.361	57	30
				0.978	4.575	3.367	4.243	4.662	26	30
					3.870	2.585	3.440	3.929	30	30
						1.262	0.924	0.108	50	30
							0.477	1.200	79	30
								0.844	91	30

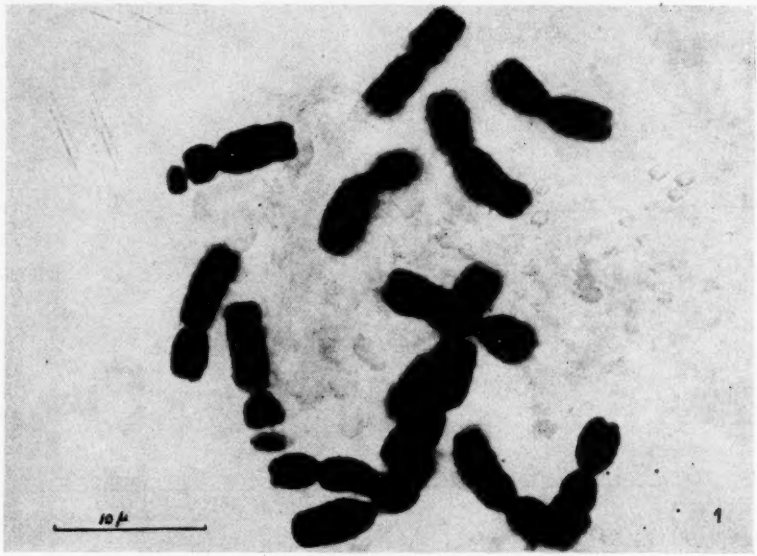
TABLE 7. *Values of »t» obtained on comparing the satellite lengths of the nine inbred lines. (From data given in Table 2.)*

No. of chrom. obs.	30	30	30	30	30	30	30	30		
Line No.	72	57	26	30	50	79	91	48		
	3.338	2.336	3.814	5.307	3.231	3.587	7.097	4.667	108	30
		0.769	0.787	3.571	7.225	0.000	4.657	1.928	72	30
			1.429	3.947	5.724	0.826	4.942	2.440	57	30
				2.667	7.382	0.855	3.724	1.129	26	30
					9.493	3.788	1.018	1.472	30	30
						7.882	10.500	7.979	50	30
							4.970	2.062	79	30
								2.484	91	30

belonging to group A with the only exception of line 26 ($\bar{x}=33.30$). This latter line (*i. e.* line 26) shows a remarkable departure from the other lines in group A, not only with regard to the length of its chromosome, but also with respect to several other characters, such as long arm length, short arm length and satellite size. This line in many respects approaches line 30, and could might as well be placed in the same group (B).

In general it can be said that lines belonging to group A have smaller chromosome lengths than those belonging to group C or B. Of them line 57 ($\bar{x}=30.07$) has the smallest (nucleolar) chromosome found in the whole series. With respect to the character »total chromosome length», it can be seen that on the whole there is a tendency in the lines to keep to the grouping originally proposed for their L.A./S.A. ratios.

This is also true in the case of the short arms. Lines belonging to group A have small short arms, whereas lines belonging to group C have large short arms. Lines 26 and 30 have short arms intermediate in size. There is a little more irregularity, when dealing with the length of the long arms. In general, there is a tendency for lines belonging to group A to have smaller long arms, than lines belonging to group C. Line 26 ($\bar{x}=19.33$) and line 30 ($\bar{x}=19.00$) also have large long arms. Line 108 ($\bar{x}=18.73$) has the largest long arms amongst lines belonging to group A, whilst line 50 ($\bar{x}=19.80$) has the same position in group C.



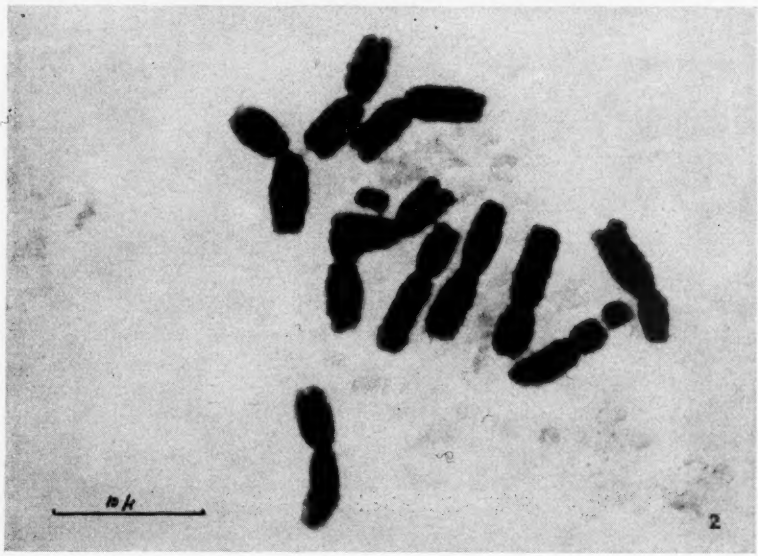


Fig. 1. Somatic metaphase of Line 108. Note the combination of small satellites and small short arms, in the nucleolar chromosomes. Oxyquinoline treated. (Note: in this and the following figures, the straight line drawn on the bottom right hand, or left hand corner of each figure, denotes a length of ten micron.) — Fig. 2. Somatic metaphase of Line 72. Oxyquinoline treated.

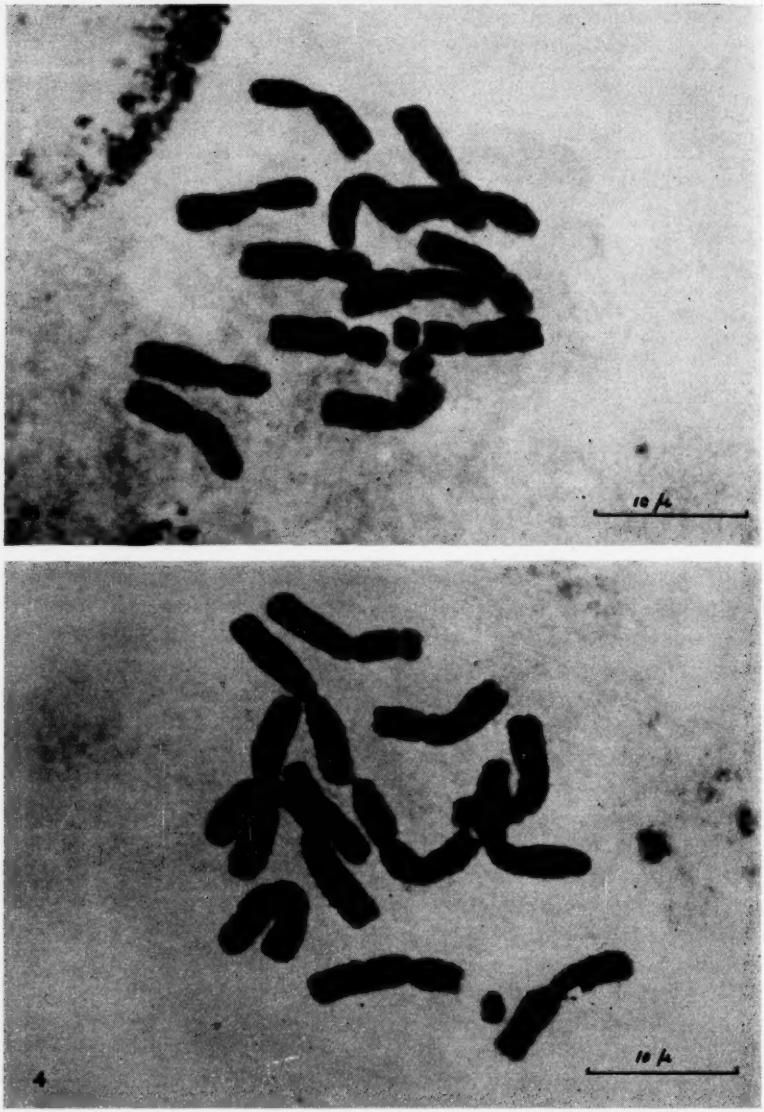
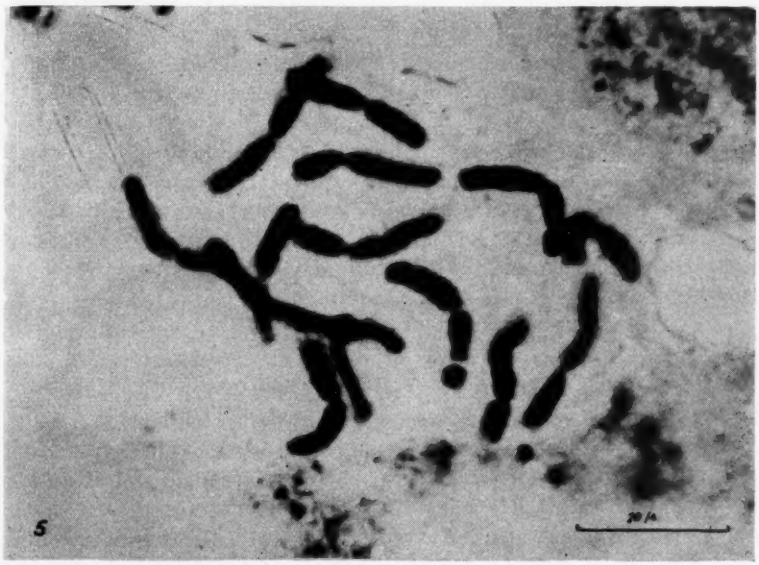


Fig. 3. Somatic metaphase of Line 30. Oxyquinoline treated. — Fig. 4. Somatic metaphase of line 91. Oxyquinoline treated. Note the combination of small satellites and large short arms. Compare with the large satellites of lines 79, 91 and 48 (*cf.* Figs. 5, 6, and 7).



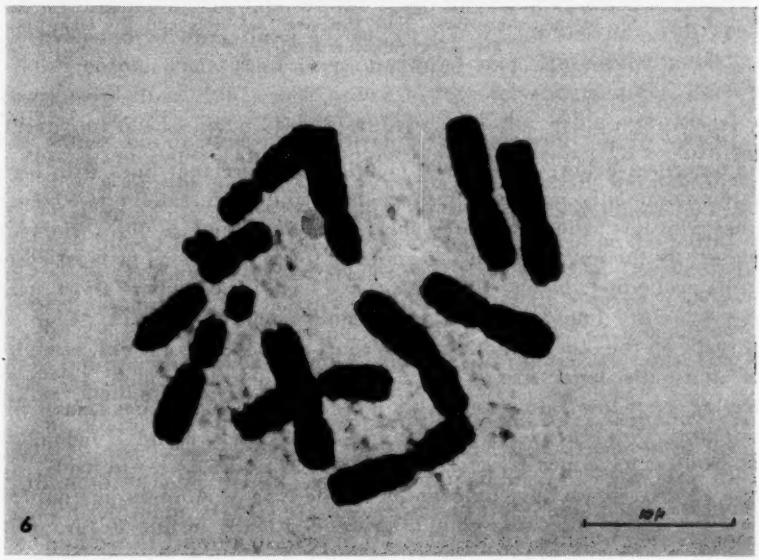


Fig. 5. Somatic metaphase of Line 79. Note the large satellites and the large short arms. Oxyquinoline treated. — Fig. 6. Somatic metaphase of Line 91. Note also the large satellites and the large short arms. Oxyquinoline treated.

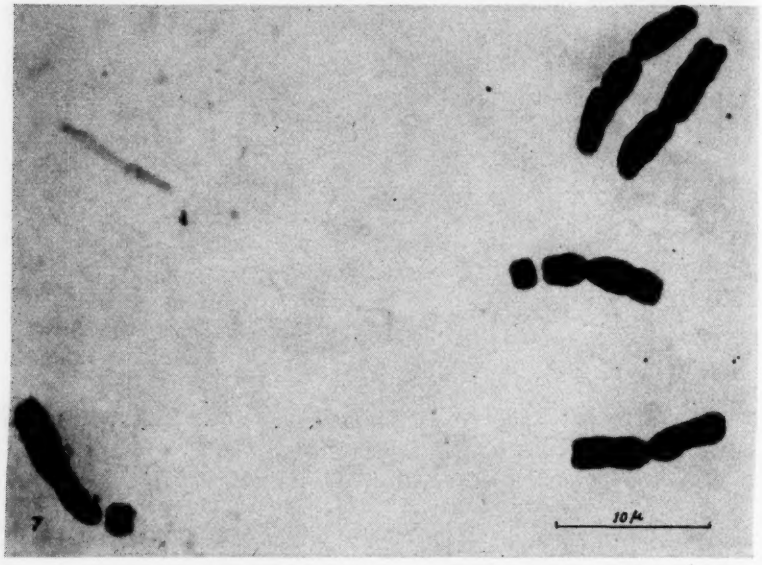


Fig. 7. Line 48. Somatic metaphase. Note the large satellites and the large short arms. Oxyquinoline treated.

In case of satellites some irregularities have been found, which will be described below. It has been mentioned before that lines with high ratios commonly have small short arms and large satellites, whereas lines with low ratios have large short arms and small satellites (the part played by the long arms in determining the ratios seems in most cases to be negligible). In line 108 (Fig. 1) we have a case where short arms ($\bar{x}=8.20$) are found together with small satellites ($\bar{x}=5.13$). Line 91 (Fig. 6) on the other hand has large short arms ($\bar{x}=10.40$) and satellites of unusually large size ($\bar{x}=6.23$). It is rather uncommon for lines having such low ratios and with large short arms, to have such big satellites. Two other lines (line 79 and line 48, Figs. 5 and 7) belonging to group C have also large satellites ($\bar{x}=5.57$ and 5.83 respectively). In line 50 (Fig. 4) we have an example of the type of chromosome pattern that is usual in lines belonging to group C. Finally in line 30 (group B, Fig. 3) we have also fairly large satellites. The other lines of group A, have as usual large satellites.

It may be of interest to note that large knobs can be distinguished in

the satellites of lines 91, 48 and 79, all of which belong to group C (*i. e.* with large short arms and low chromosome ratios) but have large satellites. It is however indiscernable in line 50, the only line in group C having small satellites (*cf.* BOSE, 1956).

As mentioned before, in Tables 3—7, are given the values of »t» as obtained on comparing the different regions of the nucleolar chromosome and the ratios of the nine inbred lines. They have been calculated from the values given in Tables 1 and 2. The figures in brackets, in the outer peripheral margin denote the total number of chromosomes that were examined in each of the inbred lines, whose number is given in the inner margin.

2. Growth and fertility studies

It was mentioned before that some connection may be found to exist, between the chromosome aberrations (as understood here) of the lines and their inbreeding behaviour. To test this hypothesis some experiments have been conducted the results of which are given below. (The author would like to thank Dr. ARNE LUNDQVIST, for allowing him access to some of his data, on the seven inbred lines presented below.)

Seven inbred lines (lines 47, 84, 76, 71, 63, 39 and 98 — of 1955) whose chromosomes had been measured and the results published previously (BOSE, 1957) were grown in four rectangular plots. Each of these four blocks had all the seven lines, each line being represented by a row of ten plants.. Thus the number of plants present in a block was $7 \times 10 = 70$ and the total number of plants sown in all the four blocks was $4 \times 70 = 280$ plants. Population plants (or F_1 hybrids obtained by crossing two inbred lines) formed the border on two sides of the block; on the other two sides (at right angles to the long axis of the rectangle) border plants were taken from the inbred lines themselves. The seven inbred lines were sown alternately in such a way that each of the seven lines had the chance of growing once next to the border, consisting of either population material or F_1 hybrids.

The characters that were examined and noted in each mature plant were (I) vegetative in nature; these included measurements of height (tallest tiller) and weight, and the total number of flowering tillers present per plant. (II) the reproductive and floral characters, including the number of flowers present in the spike of the tallest tiller, the number of kernels obtained in such a tiller and the per cent seed set.

The results of these observations have been summarised in Table 8. In the first column is given the number of the line and in the second

TABLE 8. *Data on some of the vegetative and floral characters of th* *even i* *some (L.A./S.A.) ratio* *Cf. Tal*

Line No. (1955)	Ratio (L. A./S. A.)	Pl. ht (in cm)			Pl. wt. (in gms)			No. of fl. tillers/plant			No.
	$\bar{X}$	$\bar{X}$	m	n	$\bar{X}$	m	n	$\bar{X}$	m	n	$\bar{X}$
47	2.49	111.74	0.480	39	5.46	0.480	38	2.00	0.141	38	60.63
84	2.47	99.08	2.929	39	1.37	0.145	38	1.10	0.156	39	39.59
76	2.38	104.85	1.972	39	2.76	0.982	39	1.41	0.114	39	46.62
71	2.12	112.60	1.364	40	10.23	0.817	40	3.15	0.217	40	61.40
63	2.11	104.93	1.257	40	10.50	0.332	40	3.35	0.244	40	61.40
39	1.88	120.33	1.288	40	15.83	1.039	40	5.33	0.331	40	66.30
98	1.85	100.18	0.799	40	10.48	0.699	40	3.85	0.238	40	68.15

column its corresponding L.A./S.A. ratio, obtained from a previous work (BOSE, 1957) In the other six big columns are given the mean, the standard error and the number of plants examined per line for each of the six characters mentioned above. The lines have been arranged according to the values of their ratios, and are divided into three groups, A, B and C. Lines belonging to group A have been placed on the top, on account of their high ratios. Lines belonging to group B having

TABLES 9 A and 9 B. *Analysis of variance (Height of tallest tiller).*

9 A

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between lines	6	13764.33	2294.06	
Within lines				
Between blocks	21	6406.45	305.07	2.010*
Within blocks	249	37789.75	151.77	$(0.01<P<0.05)$
Total	276	57960.53		

9 B

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between blocks	3	679.66	226.55	
Within blocks				
Between lines	24	19491.12	812.13	5.351***
Within lines	249	37789.75	151.77	$(P<0.001)$
Total	276	57960.53		

...even inbred lines (1955), together with their corresponding chromo-... (*Cf.* Tables 1 and 3, BOSE, 1957.)

No. fl. (tallest spike)			No. of kernels (tallest spike)			% seed set (tallest spike)			Group
$\bar{X}$	m	n	$\bar{X}$	m	n	$\bar{X}$	m	n	
60.63	1.761	38	34.89	1.817	38	55.98	2.379	38	A
39.59	1.664	39	12.82	1.581	39	33.18	3.792	39	
46.62	1.044	39	24.03	1.709	39	51.20	2.542	39	
61.40	0.917	40	46.15	1.249	40	74.86	1.367	40	B
61.40	0.860	40	40.85	1.175	40	66.24	1.378	40	
66.30	1.497	40	47.70	2.052	40	70.55	2.184	40	C
68.15	0.949	40	54.85	1.153	40	80.57	1.204	40	

medium ratios have been placed in the middle, and lines having low ratios have been placed at the bottom (group C).

It will be seen that there is also a general tendency for the various vegetative and reproductive characters to follow this grouping. Thus it can be seen that lines belonging to group A have less vigour than line B or C, as measured by the six characters. Lines of group B, have intermediate vigour and lines belonging to group C have the highest vigour.

TABLES 10 A and 10 B. *Analysis of variance* (*Weight of plants*).

10 A

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between lines	6	6122.44	1020.41	
Within lines				
Between blocks	21	989.90	47.14	2.793**
Within blocks	247	4169.46	16.88	(0.001<P<0.01)
Total	274	11281.80		

10 B

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between blocks	3	51.34	17.11	
Within blocks				
Between lines	24	7061.00	294.21	17.430***
Within lines	247	4169.46	16.88	(P < 0.001)
Total	274	11281.80		

TABLES 11 A and 11 B. *Analysis of variance (No. of flowering tillers).*

11 A

Course of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between lines	6	525.26	87.54	
Within lines				
Between blocks	21	89.89	4.28	2.503**
Within blocks	248	423.21	1.71	0.001<P<0.01
Total	275	1038.36		

11 B

Course of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between blocks	3	3.80	1.27	
Within blocks				
Between lines	24	611.35	25.47	14.89***
Within lines	248	423.21	1.71	(P < 0.001)
Total	275	1038.36		

TABLES 12 A and 12 B. *Analysis of variance (No. of flowers/tallest spike).*

12 A

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between lines	6	26327.97	4388.00	
Within lines				
Between blocks	21	3220.57	153.36	2.674**
Within blocks	248	14225.66	57.36	(0.001<P<0.01)
Total	275	43774.20		

12 B

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between blocks	3	298.25		
Within blocks				
Between lines	24	29250.29	1218.76	21.248***
Within lines	248	14225.66	57.36	(P < 0.001)
Total	275	43774.20		

TABLES 13 A and 13 B. *Analysis of variance (No. of kernels/tallest spike).*

13 A

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between lines	6	50737.01	8456.17	
Within lines				
Between blocks	21	5197.34	247.49	2.962**
Within blocks	248	20722.65	83.56	0.001<P<0.01
Total	275	76657.00		

13 B

Cause of variation	D. F.	Sum of squares	Mean square	Quotient (F)
Between blocks	3	353.08	117.69	
Within blocks				
Between lines	24	55581.27	2315.89	27.715***
Within lines	248	20722.65	83.56	(P<0.001)
Total	275	76657.00		

In other words lines belonging to group C, and having low ratios, seem to be more resistant to inbreeding depression.

In Tables 9—13 are given a series of variance analyses made in order to ascertain if the four blocks differed from each other significantly, and also to see if the seven lines were really different from one another.

TABLE 14. *Values of the variance ratios (F) for the 7 inbred lines (between blocks).*

D. f. = degrees of freedom; the smaller number belongs to the smaller mean square (between blocks) and the larger number to the larger mean square (within blocks).

Line No. (1955)	Ratio (L.A./S.A)	Pl. Height		Pl. Weight		No. of (fl) tillers		No. of flowers		No. of kernels	
		D. f.	F	D. f.	F	D. f.	F	D. f.	F	D. f.	F
47	2.49	3/35	2.817	3/34	6.901	3/34	5.889	3/34	9.344	3/34	10.452
84	2.47	3/35	0.409	3/34	0.212	3/35	0.600	3/35	1.263	3/35	0.695
76	2.38	3/35	3.380	3/35	1.785	3/35	2.457	3/35	1.960	3/35	1.292
71	2.12	3/36	3.401	3/36	7.806	3/36	6.492	3/36	3.406	3/36	5.340
63	2.11	3/36	0.775	3/36	1.394	3/36	1.050	3/36	0.621	3/36	0.821
39	1.88	3/36	2.106	3/36	0.602	3/36	0.548	3/36	0.932	3/36	3.070
98	1.85	3/36	3.663	3/36	5.309	3/36	4.870	3/36	0.184	3/36	0.621

Five of the six characters mentioned in table 8, were analysed. The first part of each of these tables (*viz.* Tables 9 A, 10 A, 11 A, 12 A, and 13 A) show that there are slight but significant differences, when comparing the blocks ($0.001 < P < 0.01$ in most cases) for each of the characters examined. The second part of each of these tables (Tables 9 B, 10 B, 11 B, 12 B and 13 B) show that there are again highly significant differences, ($P < 0.001$) between the lines for each of these five characters.

In Table 14 is given the variance ratios obtained for the blocks for each of the five characters of the seven inbred lines. It can be seen that the variance ratio (F) is consistently high in some of the lines (*e. g.* line 47 and line 71) in all the characters studied, indicating a high variability in these two lines. In contrast to them, some of the other lines like line 84, show little or no variability. A close examination has revealed the fact that both the lines (47 and 71) show a marked depression when grown next to population or hybrid plants, that is they show marked border effects. This can be shown if we take a set of characters like (a) plant height, (b) plant weight, (c) number of flowering tillers and compare the mean of these characters for each of the four blocks. Thus for line 47, (a) $\bar{x}=112.90$, 96,90*, 120.60, and 117.11 respectively. For the character (b), $\bar{x}=5.40$, 2.50*, 7.40, and 6.33 respectively. For (c), $\bar{x}=2.20$, 1.11*, 2.40 and 2.22 respectively. (The border rows are indicated by asterisks.) The corresponding values for line 71 are as follows, (a) $\bar{x}=115.50$, 114.30, 105.50*, and 114.90 respectively; for (b) $\bar{x}=13.70$, 12.30, 5.30*, and 9.60 respectively; for (c) $\bar{x}=3.90$, 3.80, 1.90*, and 3.00 respectively. It is clear from the above data that when plants of both these lines grow next to population plants, there is a marked fall in the values of these characters, *i. e.* they become weaker.

In contrast to the above two lines, line 63 can be taken as an example of a line having smaller border effects. The corresponding values of the four characters studied in this line are, (a) $\bar{x}=105.90$, 107,70*, 102.70, and 103.40 respectively; (b) $\bar{x}=12.60$, 7.80*, 10.40, and 11.20; (c) $\bar{x}=3.90$, 2.70*, 3.30, and 3.50.

It can be seen from the above data, that the significant difference found when comparing the blocks is really due to the border effects shown by the two lines 47 and 71. This observation, together with the fact that the values obtained for F, when comparing the lines, show such high values (with regard to all the characters studied), in contrast to the much lower values of F obtained on comparing the blocks, that

TABLE 15. *Data on some of the vegetative and floral characters of nine inbred lines (1957) that were examined, together with their respective chromosome (L.A./S.A.) ratios.*

Line No. (1957)	Ratio L.A./S.A.	Height of plants (cm)			Wt of plants (gms)			No. of (flowering) tillers			No. of kernels (tallest spike)		
		$\bar{X}$	m	n	$\bar{X}$	m	n	$\bar{X}$	m	n	$\bar{X}$	m	n
108	2.44	124.17	1.353	21	15.42	1.539	21	4.30	0.333	20	56.10	2.760	21
72	2.35	85.60	2.140	21	12.58	1.421	21	7.10	0.525	21	28.62	1.987	21
57[1]	2.24	—	—	—	—	—	—	—	—	—	—	—	—
26	2.20	77.27	1.170	21	9.91	0.995	21	6.57	0.519	21	32.90	1.643	20
30	2.10	126.31	1.285	21	18.56	1.407	20	7.71	0.594	21	42.08	1.637	21
50	1.96	117.74	1.455	21	14.45	1.407	21	6.19	0.505	21	39.71	2.419	21
79	1.93	91.98	1.260	19	15.10	1.921	19	5.84	0.563	19	38.21	2.057	19
91	1.92	99.88	1.975	21	12.74	0.970	21	5.48	0.356	21	40.10	3.324	21
48	1.88	99.40	1.220	21	13.52	1.330	21	8.10	0.801	21	33.19	1.910	21

[1] Plants of this line were extremely weak and few in numbers.

it seems permissible here to ignore the small differences obtained for the blocks.

In table 15 are given some measurements of the lines, whose chromosomes were studied in the previous section. At first sight it seems that the results obtained are highly irregular and in some cases seem to contradict the earlier interpretation. These data should be treated with caution however, because these lines were not sown in a planned way, some of the lines were grown as far apart as ten to fifteen metres or even more.

From Table 16 it can be seen that while some of the lines behave in accordance with the group interpretation given in Table 14, other lines like line 91, 108 and 29 apparently do not. This may partly be due to the differences in the environmental condition, but it may also depend on other causes, which will be taken up in the next section.

V. DISCUSSION

The main cause of inbreeding degeneration, is undoubtedly to be found at the genic level (*cf.* MÜNTZING, 1945). But at the same time it is possible that cytological factors like chromosome aberrations may also play an important part. Any aberrations in the nucleolar chromosomes, on account of their special relationship with the nucleolus, may also have some role to play, and it has been one of the object of this work, to find out, if any such connection could be established.

The difficult nature of the problem will be understood when one considers the many factors that have to be taken into account. It is not possible for instance to estimate the vigour or vitality of a plant, from one character alone. For this reason several characters have to be taken into account, as has been done with these lines (Tables 8 and 14). Even then difficulties may arise when one has to decide for example between weight and height, in a case where one line has superior height but less weight than the other line.

To reduce experimental errors, large number of plants and even lines have to be examined with proper statistical technique. This needs of course a lot of time and work. Unfortunately, mainly due to a lack of time, the experiments had to be confined to a small scale, consisting only of a few lines. It is possible that owing to the fact that only a small number of lines have been investigated, the relationship established in Table 8, between the chromosome groups and vigour of lines, may not hold good. Nevertheless, it seems promising enough to the author for

making large scale experiments, to test the possibility of the existence of such a connection.

When comparing lines, at the chromosome level, it has often been found that several regions of the chromosome may vary at the same time. Part of the variation may be due to bad technique in squashing, and in measurements, but there is no doubt, that there are differences present. In these reports, variation in the lengths of the short arms and satellites have been mainly stressed. Long arm lengths do differ a little in the different lines, but on the whole there seems to be a lack of regular pattern in this variation. At any rate variation in the L.A./S.A. ratios of the lines seems to depend more on the variation present in short arms, than on the long arms.

Similar aberrations present in the other chromosomes of the complement, have been totally disregarded here, but they may quite well affect the vigour of lines. There may also be present such types of aberrations as deletion inversions and interchanges, as have been found in some inbred lines of rye, by REES (1955). The effect of some of the aberrations may also be obscured by the interaction of genes (epistasis). Many of these factors have to be taken into account when trying to consider a hypothesis, like the one attempted below.

It has been pointed out before that certain chromosome patterns tend to occur more regularly than others (*e. g.* in population plants — BOSE, 1957). One of the types has high L.A./S.A. ratios, small short arms and large satellites (aS/aS). This type is identical with group A in Tables 1 and 2. Another common type has small L.A./S.A. ratios, large short arms and small satellites (As/As — group C). It can be seen from Tables 8 and 15, that lines having group A ratios are weaker than group C lines, provided this special type of pattern (*i. e.* aS/aS) in A, is kept. Again group C lines are more vigorous than (the normal) group A lines, whenever they keep their (As/As) type of pattern. There are lines, however, which do not have such regular pattern, though they may belong to a particular group, owing to its special type of ratio. Line 108, (Table 15) seems to be such a line. It can be seen that it has rather small satellites (Table 8, Fig. 1) for a line belonging to this group; and it differs from the other lines in the group, in being very vigorous. In group C, lines 91, 79 and 48 all have rather large satellites, for lines belonging to this group, and they are on the whole weaker than line 50, which has the normal As/As type of chromosome pattern.

All the lines given in Table 8, have rather typical chromosome patterns, characteristic for each of their groups, and it is probably on

account of this, that these groups show such clear consistent differences in growth behaviour.

It should be mentioned also that the position of lines belonging to group B is a little unclear. In Table 8 they occupy an intermediate position, with respect to vigour, but in Table 15, line 29, the only line with an intermediate ratio, has rather high vigour.

In conclusion the author would like to point out, that though some of the results obtained so far seem to be rather promising, more work, conducted on the same line and on a much larger scale is needed, before a definite answer can be given as to whether the interpretation given above, is correct or not.

SUMMARY

(1) Detailed measurements of the nucleolar chromosomes of nine more inbred lines have been presented here.

(2) It has been possible to arrange these lines according to the values of their ratios, obtained, by dividing the long arms of the nucleolar chromosomes, by their short arms (excluding the satellite regions).

(3) It has been found, that four lines (lines 108, 72, 57 and 26) can be included in the first goup A, all of them having their L.A./S.A. ratios greater than 2.2.

(4) In the second group B, has been included only one line, with a ratio of 2.1.

(5) In the third group C, four lines (lines 50, 79, 91 and 48) have been included, all having ratios less than 2.0.

(6) Experiments were set up to demonstrate the possibility of some kind of relationship existing between chromosome types of the different lines (belonging to different groups) and their inbreeding behaviour. The results of these experiments have been presented and discussed.

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HETEROPLOIDY AND POLYMORPHISM IN SOME APOMICTIC SPECIES OF POTENTILLA

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I. INTRODUCTION

IN the large and very polymorphic genus *Potentilla* several species have apomictic seed formation, but the degree of this apomixis varies in different species. Thus, for instance, in the Scandinavian representatives of the collective species *P. argentea* no trace of sexuality has so far been detected, all crosses giving only maternal offspring and progenies of single individuals being always completely uniform (MÜNTZING, 1928, 1958 a, A. and G. MÜNTZING, 1941). Other members of the *argentea*-group, from Central Europe, also have an apomictic mode of seed formation, but RUTISHAUSER (1948), using a biotype of *P. argentea* var. *demissa* as female parent, succeeded in raising true hybrids after pollination with two biotypes of *P. verna* and one biotype of *P. canescens*. These hybrids, however, were all »triploids», the result of fertilization of unreduced embryosacs of the *argentea* parent. Other types of *argentea* may be quite or almost quite sexual like the diploid biotype A—C. In this strain, which systematically probably belongs to *P. argentea* var. *calabra* SER, occurring in the Mediterranean region, most of the embryosacs are reduced but some of them are unreduced (*cf.* HÅKANSSON, 1946, pp. 27—31). In some crosses only the reduced egg cells give viable seeds, in other crosses the unreduced ovules produce all or most of the offspring (A. and G. MÜNTZING, 1945; MÜNTZING, 1958 b). Thus, in the *Potentilla argentea*-group there is quite a range from complete apomixis to almost complete sexuality.

In other related groups of *Potentilla*, the *Collinae* and *Aureae vernales*, there is also apomixis but apparently not to the same extreme degree as is characteristic of the Scandinavian *argentea* types. Thus, RUTISHAUSER (1943 a, b, c, 1945, 1948, 1949) obtained fertilization of unreduced egg cells not only in *P. argentea* but also in *P. praecox*, *P. canescens* and different biotypes of *P. verna*.

A. and G. MÜNTZING (1943), working with *P. collina*, did not produce any hybrids with this species but observed the spontaneous occurrence of changes in chromosome number. Thus, in a certain biotype, C—A, which is hexaploid ($2n=42$), a plant with $2n=84$ appeared, which gave rise to a new strain with this doubled chromosome number. The plants with 84 chromosomes are weaker than the normal C—A plants but have some gigas properties. With regard to mode of meiosis and pollen fertility a great contrast exists between normal C—A and the gigas strain, the latter having excellent pollen fertility and an almost regular meiosis, whereas normal C—A has poor pollen fertility and a very irregular meiosis. The most striking property of the 84-chromosome strain is its ability to produce a considerable proportion of reversion plants in each generation. These plants have arisen by parthenogenetic development of reduced egg cells and were found to have chromosome numbers ranging around 42. Some of them are exact hexaploids but others are aneuploids with chromosome numbers slightly deviating from 42.

Plants with doubled chromosome numbers were not only observed in C—A but also in two other strains of *P. collina*; but further work was considered necessary in order to decide whether these plants were true »tetraploids» and not intermixtures from the 84-chromosome strain of C—A, cultivated at the same time. During the 15 years that have elapsed since this paper was published (A. and G. MÜNTZING, 1943), new data have accumulated not only with regard to chromosome doubling and reversion in *P. collina* but also with regard to spontaneous aneuploidy and polyploidy in two other related species of *Potentilla*, *viz. Crantzii* BECK and *Tabernaemontani* ASCHERS. These two species units are well separated in Sweden. They are closely related to *P. verna*, which belongs to the material studied by RUTISHAUSER. The three species studied by the present writer will be dealt with separately.

II. SPONTANEOUS CHROMOSOME DOUBLING AND REVERSION IN POTENTILLA COLLINA

1. Strains with increased chromosome numbers

A. The 84-chromosome strain derived from C—A

Progenies of C—A plants with 84 chromosomes have been raised five times, the total number of plants in these progenies amounting to 93.

Of these plants, 78 represented the maternal type, and 15 were reversion plants with ± 42 chromosomes, which regularly appeared if the progenies were sufficiently large. The percentage of reversion plants, 16.1, is a minimum value as 7 plants in one of the progenies were excluded. These plants were indistinguishable from normal C—A and were probably intermixtures caused by erroneous field planting. Some of them, however, may have been true reversions plants and in such a case the average percentage of reversion would be somewhat higher than 16. The maternal plants in all these progenies appeared to be identical and only the reversion plants were dissimilar inter se.

The apomictic mode of seed formation in the 84-chromosome strain of C—A is also evident from various crosses undertaken with this strain as the female parent. Thus, in the cross 84-chromosome C—A $\times$ normal C—A ($2n=42$), 6 individuals were obtained, 3 of which were maternals with $2n=\pm 84$ and 3 reversions with ± 42 and 41 chromosomes. Crosses were also undertaken between 84-chromosome C—A and T—B, a biotype of the related species *P. Tabernaemontani*, having $2n=84$ like the other parent. In spite of the identical chromosome number this cross also gave only maternal plants, 7 individuals having ± 84 and one plant being a reversion with $2n=42$.

In one of the progenies, comprising 39 maternal plants and 6 reversions pollen fertility was examined. In the maternals the percentage of good pollen ranged from 70 to 100 with an average of 87.1. In the reversions there was a range from 10 to 50 with an average of 31.7. Thus, these observations verify the preliminary evidence (A. and G. MÜNTZING, 1943, p. 458) of a markedly higher pollen fertility in the 84-chromosome plants than in the reversions, which are as sterile as the original C—A strain.

B. New cases of increased chromosome numbers in C—A

Three new cases have been observed in which the otherwise strictly apomictic and hexaploid biotype C—A has produced deviating individuals with an increased number of chromosomes. The first of these plants, *535 e—11*, had 11 normal sister plants. It appeared to be triploid rather than tetraploid in relation to the mother biotype, and this view was supported by the finding that only 50 per cent of the pollen grains were good, a value reached by several of the normal sister plants.

A progeny of 14 plants was raised after open pollination of 535 e—11. All these plants were alike and had obvious gigas properties. Chromosome counts were made in a total of 16 plants, 2 plants having died

before transplantation to the field. Six plants were found to have $2n=65$, 8 plants ± 65, 1 plant ± 66 and 1 plant 71 to 74 chromosomes. The last-mentioned plant had died early and was not observed in the field. The result indicates a predominantly apomictic mode of seed formation, though one plant must have resulted from fertilization of a reduced embryosac. The mother plant must have had $2n=65$, being hypertriploid in relation to the normal chromosome number of the biotype. It must have arisen by fertilization of an unreduced egg cell.

The second case is represented by plant *535 c—10*, being apparently »tetraploid» and having better pollen (70 per cent good pollen) than the 11 normal sister plants. A progeny of 14 plants were raised after open pollination of this plant, nine of the plants being of gigas type and 5 plants obvious reversions. In the former category the chromosome counts gave the following result (number of plants in brackets): $2n=84$ (2), ± 84 (4), ± 83 (2), ± 82 (1). In the reversion plants the chromosome numbers were: 43 (1), ± 43 (1), 42 (1), ± 40 (2). Thus, this represents a new clear case of spontaneous chromosome doubling, the chromosome number of 535 c—10 most probably being $2n=84$.

The third case involves plant *17—19*, which was a deviating and apparently polyploid plant in relation to 23 normal sister plants of the C—A biotype. Consequently, a progeny after open pollination was raised. This progeny consisted of 12 plants, which were uniform and clearly deviated from normal C—A by having coarser dimensions. Chromosome counts were made in 10 plants, the following result being obtained: $2n=70$ (7), ± 70 (2), ± 71 (1). Thus, the chromosome number of this new strain is hypertriploid in relation to normal C—A and happens to represent an even multiple of seven. The mother plant must have arisen by fertilization of an unreduced egg cell, the male gamete in this case having 28 chromosomes. In view of the very irregular meiosis observed in normal C—A (A. and G. MÜNTZING, 1943, p. 455), the formation of gametes with such a chromosome number is quite likely.

C. Cases of spontaneous increase in chromosome number in other biotypes of P. collina

In the previous paper (A. and G. MÜNTZING, 1943, pp. 454—455) it was mentioned that single plants with 84 instead of 42 chromosomes had not only been met with in C—A but also in two other biotypes of *P. collina* from Vickleby and Resmo on the island of Öland. It was suspected that these plants might have been intermixtures of »tetra-

ploid» C—A, cultivated in the same year. However, at least with regard to »*collina* Resmo», it is now clear that the chromosome doubling was genuine. A progeny of 10 plants was raised from the deviating plant in question, and of these plants one was a reversion with $2n=\pm 41$ and with a specific appearance, the other 9 plants having about 83 chromosomes [± 84 (3), ± 83 (3), $\pm 82+1$ f (1) and ± 82 (1)] and representing a special biotype clearly different from »tetraploid» C—A. There also seems to be a difference in pollen fertility, the 84-chromosome biotype of *collina* Resmo only having about 50 per cent good pollen whereas the corresponding value in 84-chromosome C—A is generally over 80 per cent. It could not be decided whether the slightly variable results of the chromosome counts among the plants having $2n=\pm 83$ represented real or only apparent differences in the chromosome constitution.

A progeny of 7 plants were also raised from the deviating plant in »*collina* Vickleby». Two of these plants represented reversions with ± 42 and ± 39 chromosomes respectively, the other plants having $2n=84$ (1), ± 84 (2) or ± 83 (2). These plants probably represented a new »tetraploid» biotype of *collina* Vickleby, although it could not be established with certainty that these plants deviated from »tetraploid» C—A.

In another case, however, the possibility of an experimental error is excluded. In the *collina* biotype C—F ($2n=42$), which has a very characteristic morphology, a single deviating plant with some gigas properties was noticed. In 1945 a progeny of this plant, »74—6», was raised; it consisted of 59 individuals. 56 of these plants were gigas types like the mother; 3 were weaker and morphologically deviating. The chromosome numbers were determined in one of the aberrants which was found to have $2n=\pm 57$ and in 35 of the gigas plants which were all found to be »triploid» with $2n=\pm 63$. More precisely, $2n=63$ was counted in 28 plants, $2n=62$ in 5 and $2n=64$ in 2 plants, but the differences between these counts are probably only apparent. At any rate, it is clear from this result that the deviating mother plant had arisen by an exceptional fertilization of an unreduced embryosac. It is also clear that this deviating plant retained most of the apomictic ability characterizing the mother biotype. This conclusion was confirmed by a second progeny of the original deviating mother plant, which was raised some years later. In this case 19 individuals were obtained, which were all maternal. Chromosome counts were undertaken in 17 of these plants, all having 62 to 64 chromosomes except one which apparently had $2n=61$. It is probable, however, that all these plants had the same

constitution although the exact chromosome number was sometimes difficult to establish. In both generations plants of normal C—F (in all 27 individuals) were cultivated at the same time as the »triploids». These progenies were quite uniform.

Pollen fertility was studied in the material raised in 1945, »triploid» C—F being found to have significantly better pollen than normal »diploid» C—F. In 14 plants of the former category the percentage of good pollen ranged from 60 to 90 with an average of 82.1; in the latter category the range in 12 plants was from 20 to 50 with an average of 30.8. The original deviating mother plant, 74—6, had been observed to have 80 per cent good pollen, whereas three normal sister plants examined at the same time had only 20 or 30 per cent good pollen. Thus, it is clear that the change in chromosome number from 42 to 63 has caused a considerable improvement in the pollen fertility. Total vigour, on the contrary, is reduced in spite of the larger flowers and leaves and the thicker stems. In 1949 plant weight was determined for a number of *collina* biotypes including C—F and C—F, gigas. In the former the average weight of 15 plants was 158.4 gr., the corresponding value of the »gigas» plants being only 96.1, this difference obviously being significant.

2. The formation of new biotypes from reversion plants

As we have seen, in strains with 84 chromosomes that had arisen by spontaneous chromosome doubling a considerable proportion of the offspring are reversion plants having approximately half the chromosome number of the mother plant. Owing to meiotic irregularities the female gametes, which develop parthenogenetically, do not always have 42 chromosomes but may sometimes represent aneuploid chromosome numbers so far observed to range from 39 to 43 (*cf.* Fig. 1).

The reversion plants deserve a careful study from several points of view. Thus, it is of interest to investigate their mode of reproduction, their vigour and fertility and the possible differences between strains derived from different reversion plants. Such studies were undertaken with regard to the C—A reversions. In 1944, a special trial was laid out in which progenies of five different reversion plants (after isolation or open pollination) alternated with plots of normal C—A. In this material chromosome numbers, pollen fertility and plant weight were determined, and morphological observations were made. The results obtained were as follows.

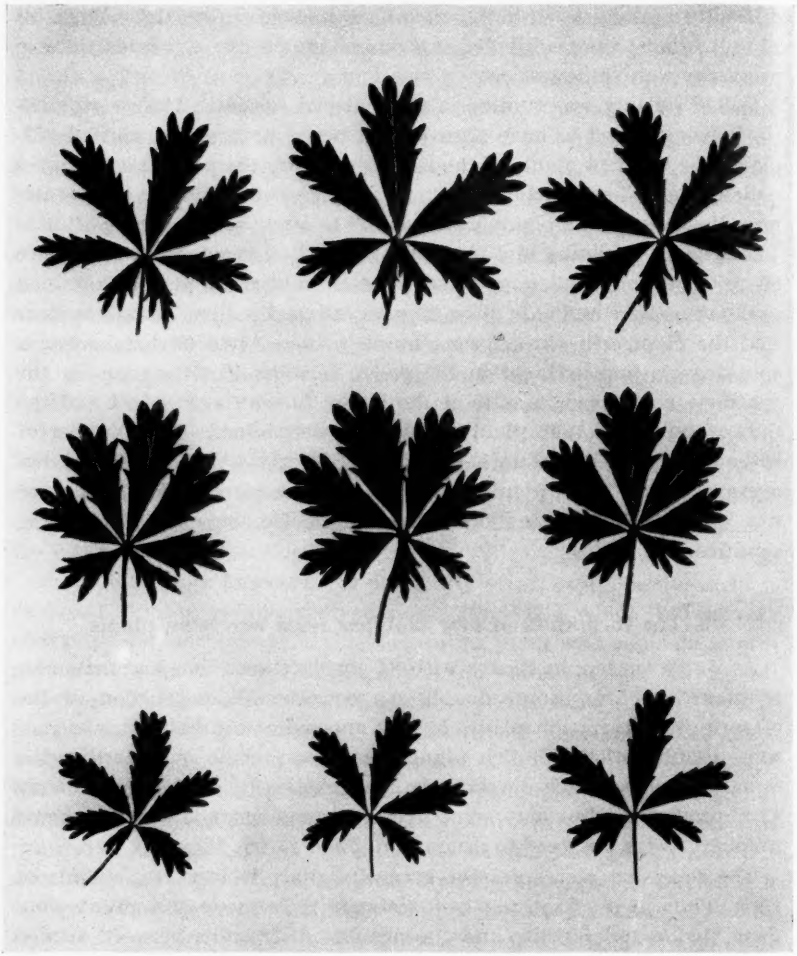

Fig. 1. Leaves of original material, chromosome doubled strain and reversion biotype in the strain C—A of *Potentilla collina*. Above, starting material, normal C—A ($2n=42$); in the middle, »tetraploid» C—A ($2n=84$); below, reversion biotype with $2n=43$.

A. Morphology and chromosome numbers

1944—518. This plot, consisting of 56 plants, represented the offspring after open pollination of plant *336—10*, having $2n=40$. Of the 56 daughter plants, 42 appeared to be »diploid», 3 »triploid» and 11 »tetraploid». (Here and in the following, »diploid» means having the normal hexaploid chromosome number of C—A ($2n=42$), »triploid» and »tetraploid», having chromosome numbers about 50 and 100 per cent higher than $2n=42$, *i. e.* ± 63 and ± 84.)

Chromosome counts were made in 17 of the diploid plants, the following result being obtained: ± 39 (1), 40 (7), ± 40 (8), ± 41 (1). This indicates cytological constancy, probably all these plants having 40 chromosomes like the mother plant. All these diploids were morphologically alike and have most probably arisen by parthenogenesis of unreduced ovules. They were similar to the mother clone, C—A, but differed by having broader leaflets and less good vigour.

Of the three supposed triploids one was cytologically examined and found to have 60 to 62 chromosomes, the chromosome count thus verifying the morphological impression. A progeny consisting of 40 individuals was raised from this plant after open pollination. This progeny was almost completely uniform, only one plant being clearly deviating. This plant was weaker and more gracile and was found to have $2n=\pm 40$. All other plants that were cytologically examined were found to be triploid, the following chromosome numbers being obtained: 61 (8), ± 61 (21), ± 60 (4), ± 62 (1). Thus, this material represents a new, almost constant strain with $2n=61$, which is in agreement with the count made in the mother plant (60 to 62 chromosomes).

Progenies were also raised from the other two plants suspected of being triploid, but in which the chromosome number had not been determined. In both cases, however, the chromosome numbers in the offspring revealed that the mother plants had been diploid. In the first one of these progenies, consisting of 25 plants, the following result was obtained: $2n=40$ (15), ± 40 (8), ± 41 (1), ± 78 (1). In the second progeny the following counts were made: $2n=40$ (4), ± 40 (5), 77 (1). A point of special interest is the occurrence in each of these progenies of a plant with doubled chromosome number, this number being more or less clearly below 80. This indicates that these tetraploids have arisen by fertilization of unreduced embryosacs with pollen grains of the same plant, which were also unreduced but which had lost a few chromosomes. This view is supported by the fact that giant and presumably unreduced pollen grains were observed to occur in the 40-chromosome

plants of this biotype as well as in some other reversion types of C—A (*cf.* Tab. 1).

Of the eleven apparent tetraploids in plot 1944—518, five were cytologically controlled and found to have ±80 (3), 79 (1) and ±79 (1) chromosomes. No clear differences could be seen between the tetraploid plants, and it is doubtful whether any of these numbers is really below 80.

The occurrence of a true triploid demonstrates that apomixis in the mother plant, although predominant, was not absolute; unreduced embryosacs in exceptional cases were fertilized. With regard to the origin of the tetraploids several different possibilities may be considered. If the chromosome numbers are really subtetraploid, as was probably the case with the two tetraploids appearing in progenies from supposed triploids, self-fertilization with approximately unreduced pollen grains must have taken place. If the chromosome numbers are not clearly below 80, other alternatives must be taken into consideration, namely a) somatic doubling at the first division of the unreduced egg cell, b) parthenogenetic development from embryosacs having twice the somatic chromosome number and c) fertilization of unreduced embryosacs with the pollen of tetraploid C—A, the mother plant being open-pollinated. In the third alternative, however, the chromosome numbers should have varied around an average value of 40+42=82 and some degree of morphological variation should also have occurred. This does not agree with the observations, and this alternative may therefore be excluded. Although the precise origin of the tetraploids is still unknown, we may conclude that the reversion plant 336—10 was quite predominantly apomictic, and that this mode of reproduction was also retained in a triploid plant exceptionally arisen by fertilization of an unreduced egg cell.

1944—520. This plot, consisting of 58 plants, represented the offspring after open pollination of plant *336—13*, having $2n=\pm 42$. No triploids could be seen in this progeny, which consisted of 51 plants considered to be diploids and 7 plants judged to be tetraploid. In the former category three or four plants were morphologically deviating, one of them being gracile and having small light-green leaves. After the morphological observations this plant was also found to be cytologically deviating, having $2n=41$, in contrast to 19 other diploid plants, 5 of which had $2n=\pm 42$ and 14 exactly $2n=42$.

Of the 7 plants considered to be tetraploid, 2 were cytologically examined and found to have 84 and 83 chromosomes respectively. Two

other plants which had died at the seedling stage had 84 and ±83 chromosomes respectively. As it is uncertain whether the tetraploids are identical or represent a slight degree of chromosome variation we cannot decide, in the present case, whether the tetraploids have arisen from fertilization of unreduced egg cells by the pollen of tetraploid C—A or from unfertilized ovules as in the previous reversion plant.

Most of the diploids are certainly identical, but at least one plant was aberrant. This individual with $2n=41$ may have arisen by fertilization of an exceptional reduced embryosac or possibly by somatic non-disjunction. Though the diploids in the present progeny have $2n=42$ like normal C—A, a difference was observed, the diploids in 1944—520 being smaller and hence growing more separated from each other than in the adjoining plot of C—A, in which the plants had a stronger tendency to grow into each other. It was also observed that leaf shape and colour in the diploids of 1944—520 were clearly different from those of the diploids in the previous plot, 1944—518. This is, of course, to be expected, the two categories of diploids differing in chromosome number ($2n=42$ and 40 resp.).

1944—521 and 1944—522. These plots represent progenies after isolation and open pollination of reversion plant *336—19*, having $2n=42$. The former progeny consisted of 18 plants, which were all judged to be diploid. This was found to be true, 17 plants having exactly 42 chromosomes, and 1 having $2n=\pm 42$. The other progeny, after open pollination, was larger, consisting of 53 plants, 45 of which were considered to be diploid, the remaining 8 to have increased chromosome numbers. Of the latter ones, 6 were judged to be tetraploid and 2 were suspected of being triploid. However, one of the suspected triploids turned out to have $2n=84$, thus being tetraploid; two of the supposed tetraploids had ±82 and ±84 chromosomes. Of the supposed diploids, 9 plants were cytologically examined, all of them having $2n=42$. These diploids were similar to normal C—A plants but appeared to be somewhat less vigorous. In the tetraploid category as well as among the diploids some variation was observed, but it is not excluded that it was due to environmental conditions rather than to genotypical differences.

1944—523 and 1944—524. Here we again have progenies after isolation and open pollination of another reversion plant, *336—27*, having $2n=42$. The former progeny (isolation) consisted of 40 individuals, which were uniform and quite similar to normal C—A plants. Twelve of these plants were cytologically examined, 11 having $2n=42$ and 1 plant ±42. In the progeny after open pollination (1944—524) there

were 49 plants, 45 of which were clearly diploid. Ten of them were cytologically examined, 9 having $2n=42$ and 1 plant ± 42. In addition to the 45 diploid plants the progeny in question also contained 4 tetraploids which were similar inter se. Chromosome counts were made in two of them, the result being ± 83 and ± 84.

It is of particular interest that the diploid offspring of this plant, 336—27, was slightly but clearly different from the diploids in the previous progenies of plant 336—19. Thus, though both these reversion plants had the normal chromosome number $2n=42$, they must have been genotypically different.

1944—526. This progeny, consisting of 55 plants, was raised after open pollination from plant 336—28, having $2n=39$. The aneuploid constitution of the mother plant was verified by chromosome counts in the offspring and also clearly influenced the vigour of the plants, which was much reduced in comparison to the adjoining standard plants of normal C—A. In this progeny there was an unusually high proportion of plants with increased chromosome numbers, 14 plants of 55 belonging to this category. Chromosome counts were made in 19 diploid plants, the result being $2n=39$ (17) and ± 39 (2). Three tetraploids were examined and found to have 78, ± 78 and ± 77 chromosomes respectively. Two other plants, which died as seedlings, were both found to have ± 77 chromosomes.

To this it may be added that a new progeny of 336—28 was raised two years later. This progeny, *1946—11*, consisted of 19 plants, 17 of which were typical, representing the maternal type, the remaining 2 plants being tetraploid. Chromosome counts were made in 18 of these plants, the two tetraploids having $2n=78$ and ± 76, the diploids having $2n=39$ (14) or ± 39 (2).

From this it is quite clear that the tetraploids cannot have arisen by fertilization of unreduced egg cells with pollen from tetraploid C—A. In such a case the result would have been tetraploids with $39+(\pm 42)$ chromosomes, and as a rule such plants should have 81 chromosomes with a certain variation around this number. As none of the tetraploids had more than 78 chromosomes, this alternative is excluded. This being the case we must instead assume that the tetraploids arose in one or more of the following ways: a) by fertilization of unreduced egg cells with unreduced pollen grains of the same plant, giving $39+39=78$; b) by parthenogenetic development of unreduced egg cells, followed by somatic doubling; c) by parthenogenetic development from egg cells having twice the somatic chromosome number.

Adding the plants of the two progenies 1944—526 and 1946—11 we get a total of 58 diploids and 16 tetraploids. This gives an average percentage of tetraploids of 21.6, which is a much higher value than in any other progeny. It is possible that the markedly aneuploid constitution of the mother plant is in some way responsible for the abnormally high proportion of tetraploids in the offspring.

1944—527 and 1944—528. These plots represented offspring after isolation and open pollination respectively from the reversion plant *336—40.* The chromosome number of this plant had not been determined.

The isolation progeny consisted of 19 individuals, 18 of which were diploid with $2n=42$ (15) or ± 42 (3). One plant was found to be tetraploid, having $2n=\pm 83$. This tetraploid was unusually vigorous but differed clearly, especially in leaf shape, from the diploids. These diploids and the corresponding ones in progeny 1944—528 were also characterized by their vigour, which clearly surpassed that of the adjoining standard plot of C—A. The petioles of these diploids seemed to be longer than in the standard.

The progeny after open pollination consisted of 35 diploids and 3 tetraploids. In the former category 10 plants were cytologically controlled, 9 having $2n=42$ and 1 plant $2n=\pm 42$. Two of the tetraploids were controlled and both found to have $2n=\pm 83$.

From these results it is clear that the chromosome number of the mother plant must have been $2n=42$ and its mode of reproduction predominantly or exclusively apomictic. It is of special interest that one of the tetraploids appeared in the progeny raised after isolation of the mother plant. This plant cannot be the result of a spontaneous cross with tetraploid C—A but must have arisen either parthenogenetically or, less likely, by fertilization of an unreduced egg cell with an unreduced pollen grain of the same plant.

B. Pollen fertility

As pointed out previously (p. 282) normal C—A is markedly pollen-sterile, whereas tetraploid C—A has much better fertility. A similar improvement in pollen fertility was also met with in the *collina* biotype C—F, when comparing normal C—F and a hypertriploid derivative (p. 285). Reversion plants from tetraploid C—A do not only revert with regard to chromosome number and morphology but also with regard to pollen fertility, the percentage of good pollen being as low or even lower than in diploid C—A.

TABLE 1. *Pollen fertility in progenies of reversion plants in P. collina (C—A).*

Field number	Chromosome number	Per cent good pollen											n	$\bar{x}$	Anthers dehisce poorly	Giant pollen grains
		0	10	20	30	40	50	60	70	80	90	100				
1944—518	40	13	24	5									42	13.1	+	+
»	61							1					1	(55.0)	—	—
»	± 80								1	2	7	1	11	82.3	—	—
1944—520	42	4	12	6									22	15.9	+	+
»	41			1									1	(25.0)	—	—
»	± 84						1	—	1	2	3		7	73.6	—	—
1944—521—522	42	2	3	11	7	4							27	28.0	+	—
» »	± 84							1	2	1	3		7	73.6	—	—
1944—523—524	42	2	—	3	10	11	1						27	36.5	+	+
» »	± 84								2	—	1	1	4	(77.5)	—	—
1944—526	39	10	9	2									21	11.2	+	—
»	± 78							2	1	4	7		14	76.4	—	—
1944—527—528	42			2	7	14	3						26	41.9	—	—
» »	± 84									2	1		3	(78.3)	—	—
Normal C—A	42		1	10	31	33	4						79	38.7		

The field trial of 1944, in which progenies of five different reversion plants of C—A alternated with plots of normal C—A, was a suitable material also for studies of pollen fertility. The occurrence of differences in fertility between various reversion types as well as differences between various levels of ploidy could be established. The data obtained are summarized in Table 1.

The first fact to be seen from the table is the very marked contrast between »diploids» and »tetraploids», the products of chromosome doubling always having much better pollen fertility than the diploids. This is especially striking in the aneuploid reversions 1944—518 and 1944—526, in which the percentage of good pollen in the tetraploids is six or seven times higher than in the diploids. In these »diploids», having $2n=39$ and $2n=40$, the average percentage of good pollen is only 11.2 and 13.1 respectively, the corresponding values of the euploid reversions, having $2n=42$, all being higher. Thus, without making a special statistical analysis it is evident that the loss of 2 or 3 chromosomes has caused a considerable reduction in fertility on the male side. However, also among the reversions having $2n=42$ there are significant differences in pollen fertility. There are four such series with the average values 15.9, 28.0, 36.5 and 41.9 (Tab. 1). The heterogeneity of these series was tested with an analysis of variance and found to be significant, the quotient between the mean squares for the variation between and within series being 32.51, which corresponds to a P smaller than 0.0005.

In the last row of Table 1 values are given for pollen fertility in normal C—A, which was used as a standard in the trial. As such, the C—A plants were divided into 7 plots distributed between the other plots of the trial. As there was no heterogeneity between the various groups of C—A plants, only the total series is given in the table. Only one of the reversion types (1944—527 and —528) gave an average value higher than that of the standard, but also 1944—523 and —524 had about the same degree of pollen fertility as normal C—A. Otherwise not only the aneuploids but also the two remaining euploid reversion types had obviously lower fertility values than the original biotype from which the reversions are derived.

It remains to point out that pollen fertility was also reduced in a quantitative way in at least 5 of the reversion types (indicated by a + in Table 1, to the right). This means that in these cases the anthers dehisced with difficulty, and that probably also the number of pollen grains per anther was considerably reduced. It should also be mentioned

that in three of these biotypes the pollen samples contained a proportion of giant pollen grains, which may be responsible for the origin of at least some of the tetraploid plants obtained.

C. Vigour and morphology

As the various reversion progenies were more or less clearly different in vigour and morphology it seemed desirable to get some numerical data proving this point. Thus, in the first place *plant weight* was determined. Excluding the tetraploid and triploid aberrants, the average plant weights in the reversion progenies ranged from 51.0 to 92.4 with a general average of 78.9 gram. The corresponding average of normal C—A was 77.1, and that of all tetraploids and a few triploids only 33.3 g. Thus, chromosome doubling in this material quite clearly causes a reduction of plant weight to less than half the value of the diploid sister plants. The loss of 3 chromosomes in progeny 426 (the plants having $2n=39$ instead of 42) also caused a clear reduction in plant weight, the average of this family, 51.0 g, being the lowest value of all the reversion progenies. Progeny 418, on the contrary, having $2n=40$ instead of $2n=42$, did not seem to suffer from the loss of 2 chromosomes, this progeny being quite vigorous (average: 85.5 g.).

An analysis of variance of the weight values in the reversion progenies (excluding tetraploids and triploids) showed a significant heterogeneity. However, the standard variety C—A, which was divided into 7 plots alternating with the reversion progenies, also showed a significant, though less pronounced heterogeneity, caused by environmental variation. Under such circumstances plant weight in the reversion progenies does not give us much information about the genotypical differentiation of this material. This may be better illustrated by characters which are less influenced by environmental disturbances such as certain leaf dimensions, which seemed to be different in different reversion progenies. Therefore the length : breadth index of the largest leaflet, the number of leaf dents of this leaflet and the number of leaflets per leaf were determined with the following results.

Leaflet index. — The results of these measurements are given in Table 2. In this case there is no significant heterogeneity between the seven plots of the standard variety C—A, the v^2 quotient between the mean squares for the variation between and within series being as low as 1.10. Excluding the tetraploid and triploid plants the six reversion progenies were analysed in the same way and found to be significantly heterogeneous, v^2 being 24.43 and P smaller than 0.0005. Now, this

TABLE 2. *Leaflet index in C—A and different reversion biotypes.*

Category	Chromosome number	Leaflet index 1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	n	$\bar{x}$
C—A, plot a	42									1	6	2	—	1	1	1					12	2.71
» , » b	»									1	3	1	2	2	2	1					12	2.79
» , » c	»										1	1	4	1	3	1					11	3.86
» , » d	»									1	1	3	—	3	2	1					11	2.82
» , » e	»									4	—	—	3	1	2	—	—	1			11	2.78
» , » f	»								1	1	—	3	3	—	4						12	2.78
» , » g	»										2	2	1	2	2	1	1	1			12	2.90
C—A, total	42								1	8	13	12	13	10	16	5	1	2			81	2.81
Reversion biotype 518	40					1	1	4	5	8	6	4	7	2							38	2.56
» » 520	42							1	1	7	13	9	7	5	5	1	—	—	—	1	50	2.72
» » 521 + 522	»										3	5	4	8	22	10	6	2	2	1	63	3.00
» » 523 + 524	»							1	3	6	11	12	15	15	19	—	3				85	2.80
» » 526	39				1	—	1	2	3	—	3	6	5	5	8	2	1	1	1		39	2.78
» » 527 + 528	42									2	2	8	7	11	17	3	2				52	2.88
»Diploid » reversion biotypes, total	39—42				1	1	2	8	12	23	38	44	45	46	71	16	12	3	3	2	327	2.81
»Tetraploid» and a few »triploid» reversion plants, total	Chiefly 78—84 a few 60—63	3	4	2	15	8	10	3	3	2	2										52	2.10

TABLE 3. *Number of leaf dents in C—A and different reversion biotypes.*

Category	Chromosome number	Number of leaf dents 7	8	9	10	11	12	13	14	15	n	$\bar{x}$
C—A, plot a	42				2	6	1	2	1		12	11.50
» , » b	»			1	—	4	2	3	2		12	12.00
» , » c	»			1	2	5	1	2			11	11.09
» , » d	»			3	3	2	1	2			11	10.64
» , » e	»			2	1	5	3				11	10.82
» , » f	»				3	6	3				12	11.00
» , » g	»				1	6	—	4	—	1	12	11.92
C—A, total	42			7	12	34	11	13	3	1	81	11.30
Reversion biotype 518	40			6	9	12	8	2	—	1	38	10.87
» » 520	42	2	1	21	13	10	2	1			50	9.76
» » 521 + 522	»			3	3	24	19	10	3	1	63	11.68
» » 523 + 524	»			4	18	32	17	11	2	1	85	11.27
» » 526	39	1	5	5	4	12	5	6	1		39	10.67
» » 527 + 528	42			7	6	26	7	5	2		53	11.06
»Diploid» reversion biotypes, total	39—42	3	6	46	53	116	58	35	8	3	328	10.97
»Tetraploid» and a few »triploid» reversion plants, total	Chiefly 78—84 a few 60—63	1	—	7	11	12	11	9			51	10.96

heterogeneity may be suspected to be due to only the aneuploid biotypes 418 and 426, the other euploid progenies with $2n=42$ possibly being homogeneous with regard to leaflet index. However, also within the 42-chromosome group there is a significant heterogeneity, v^2 in this case being 23.01 and P again smaller than 0.0005.

Finally, it may be seen from Table 2 (below) that in the aberrants with triploid or tetraploid chromosome number the leaflet index is much lower than in normal C—A and in the diploid reversion biotypes, the values in 52 plants measured ranging from 1.7 to 2.6 with an average of 2.10. This characteristic feature of the polyploids, to have relatively broader leaflets than the diploid original material is here too obvious to need any statistical treatment (*cf.* Fig. 1).

Number of leaf dents. — The results of the counts are given in Table 3. The variation between the seven different plots of the original

TABLE 4. *Number of leaflets in C—A and different reversion biotypes.*

Category	Chromosome number	Number of leaflets 5	6	7	8	n	$\bar{x}$
C—A, plot a	42	1	2	9		12	6.67
» , » b	»	2	1	9		12	6.58
» , » c	»	1	1	9		11	6.73
» , » d	»	2	5	4		11	6.18
» , » e	»	3	1	7		11	6.36
» , » f	»	4	4	4		12	6.00
» , » g	»	4	—	8		12	6.33
C—A, total	42	17	14	50		81	6.41
Reversion biotype 518	40	5	7	26		38	6.55
» » 520	42	3	5	42		50	6.78
» » 521 + 522	»	5	7	51		63	6.73
» » 523 + 524	»	4	9	72		85	6.80
» » 526	39	30	5	3		38	5.29
» » 527 + 528	42	2	8	43		53	6.77
»Diploid» reversion biotypes, total	39—42	49	41	237		327	6.41
»Tetraploid» and a few »triploid» reversion plants, total	Chiefly 78—84 a few 60—63	11	12	27	1	51	6.35

biotype C—A was found to be insignificant, the quotient between the mean squares for the variation between and within series being 2.06, which corresponds to a P value intermediate between 0.2 and 0.05. Excluding the tetraploid and triploid plants the six reversion progenies were found to be significantly heterogeneous, the quotient between the mean squares for the variation between and within series being 13.96, which corresponds to a P smaller than 0.001. The same degree of significance is obtained if the aneuploid biotypes 520 and 526 are excluded and the test of heterogeneity is limited to the four euploid biotypes having $2n=42$. In this case the quotient was found to be 26.02 and $P < 0.001$.

The tetraploid and triploid aberrants did not deviate from the diploids in regard of number of leaf dents, the average of the summarized series of the former category being 10.96 and that of the latter category 10.97 (Table 3).

Number of leaflets. — The data obtained with regard to this character are given in Table 4. Reversion biotype 526 deviates from all the other biotypes by having, as a rule, only 5 leaflets per leaf, the average value being 5.29. Also in the tetraploid plants of 526 there is a preponderance of leaves with 5 or 6 leaflets. In all other biotypes most leaves have 7 leaflets, though a variable proportion have only 6 or 5. The heterogeneity of this material was tested with a χ^2 analysis, using only two classes, one consisting of plants with 7 leaflets, the other of plants with 5 or 6 leaflets. In the original variety C—A the number of individuals in the seven plots was too low to allow a χ^2 test of the kind indicated. In the six reversion progenies, however, a significant heterogeneity was found, χ^2 being as large as 93.71, which corresponds to a P value much smaller than 0.001. This heterogeneity was chiefly caused by the sharply deviating values of progeny 526, which consists of aneuploid plants having $2n=39$. Also the other aneuploid, progeny 518, having $2n=40$, has a lower average value than the four euploid reversion types having $2n=42$. Among these biotypes there was no significant heterogeneity, χ^2 being as small as 0.52.

Conclusions. — The data given in Tables 2 and 4 and discussed above demonstrate that reversion biotypes, representing reversions from the doubled chromosome number 84 to the normal number, 42, of the original strain C—A, or to chromosome numbers slightly higher or lower than 42, are significantly heterogeneous. This was found to be true with regard to leaflet index, number of leaf dents and number of leaflets. In contrast to the reversion progenies seven plots of normal C—A, alternating in the field with the reversion progenies, did not show any significant heterogeneity. Thus, the reversion progenies are at least partly different *inter se* and consequently some of them at least also differ from the original strain, C—A. The deviations among the reversion progenies are most pronounced in the aneuploid reversions, but significant differences were also found between the euploid reversions. The plants with increased number of chromosomes (generally tetraploids) found in the reversion progenies differed from the diploid sister plants in regard of leaflet index but not in regard of number of leaf dents nor in number of leaflets.

3. Crossing experiments involving normal C—A and its chromosome-doubled and reverted derivatives

Crosses were undertaken in order to test whether the reversion types having a changed genetical constitution would be more sexual than

normal C—A. Crosses were also made between tetraploid and diploid C—A and between tetraploid and reverted C—A in order to produce, if possible, triploid hybrids. The following results were obtained:

C—A reversion×*C—A*. The maternal plant in this cross was 336—28, having $2n=39$. As the chromosome number of C—A is $2n=42$, true hybrids would generally have other chromosome numbers than 39. However, of the 52 plants raised from this cross combination, 49 were obviously maternal; and in 44 plants this was verified by chromosome counts. Thirty of these plants were found to have $2n=39$; in 12 plants the result was $2n=\pm 39$, in one plant ± 40 and in another plant ± 38. In the latter two plants the deviations from 39 are most probably only apparent. The remaining three plants in the progeny appeared to represent cases of chromosome doubling and were indeed found to have $2n=\pm 78$ (1 plant) or $2n=\pm 76$ (2 plants). Thus, in spite of its changed chromosome number, the mother plant is just as strictly apomictic as normal C—A, not a single hybrid being obtained. This is in agreement with the constancy observed in progenies of 336—28 raised after open pollination (*cf.* above p. 290). In these progenies, however, there was a higher proportion of chromosome-doubled plants, the ratio obtained being 58 diploids to 16 tetraploids. In the progeny now under discussion the corresponding ratio was 49 : 3. As may be controlled by a χ^2 test, the probability that these two ratios represent the same statistical population is intermediate between 0.02 and 0.01. The cause of this difference is not known.

C—A reversion×*C—F*. An attempt was also made to cross reversion plant 336—40 ($2n=42$) with another *collina* biotype, C—F. A total of 50 plants were raised from this cross but not a single deviating individual could be detected, the whole progeny probably being strictly maternal. In this case both parents have $2n=42$, and the same number was found in 47 maternal plants cytologically examined (38 plants had $2n=42$, 7 plants ± 42, 1 plant ± 43 and 1 plant ± 41). Thus, neither in this cross was there any indication of sexuality in the reversion plant.

C—A reversions×*tetraploid C—A*. The three reversion plants, 336—19, 336—27, 336—40, all having $2n=42$, were used as female parents. The cross 336—19×tetraploid C—A resulted in only three plants, two of which were maternal with 42 and ± 42 chromosomes, and one tetraploid with $2n=83$. The latter plant may represent a true hybrid, an unreduced embryosac of the mother plant being fertilized by a reduced male gamete. This is uncertain, however, as tetraploid plants also

occurred in the offspring of 336—19, raised after open pollination (*cf.* above p. 290).

Crosses between reversion plant 336—27 and tetraploid C—A gave a total of 12 plants, 11 of which were maternal with $2n=42$ (10) or ± 42 (1), and 1 plant tetraploid with $2n=\pm 82$. In the crosses with 336—40 a total of 33 plants were obtained, 31 of which were maternal and 2 tetraploid. One of the tetraploids had $2n=\pm 83$, the other a high chromosome number which could not be accurately determined (bad slide). Chromosome counts were also made in 17 of the maternal plants with the following result: $2n=42$ (11), ± 42 (5), $\pm$ 43 (1).

For the same reasons as before it cannot be decided whether the tetraploid plants obtained in the two latter crosses were true hybrids or the result of spontaneous chromosome doubling independent of the crosses.

C—A×tetraploid C—A. Not only reversion plants but also normal C—A were hybridized with tetraploid C—A. From two different crosses of this kind, giving similar results, a total of 47 plants were obtained. From morphological inspection alone, 8 or 9 of the plants were considered to be tetraploid, and this was verified for 7 of them, in which the chromosome counts gave the following results: ± 84 (1), 83 (1), ± 82 (4), ± 81 (1). The remaining plants were apparently maternal, and in 33 plants this was verified by chromosome counts, the plants having $2n=42$ or ± 42 (in one case ± 41).

As spontaneous chromosome doubling in C—A is certainly much less frequent (*cf.* pp. 282—283) than the frequency of tetraploids in the present progenies, we must conclude that some unreduced egg cells of C—A were really fertilized by reduced male gametes of tetraploid C—A. This being the case, we may also conclude that all or some of the tetraploid plants obtained in the crosses C—A reversions×tetraploid C—A may have arisen in the same way.

In this connection it should also be recalled that the few individuals obtained from the reciprocal cross, tetraploid C—A×normal C—A described above (p. 282), did not represent any true hybrids but only maternal offspring resulting from the parthenogenetic development of unreduced or reduced egg cells.

III. HYBRIDS BETWEEN THE COLLINA BIOTYPES C—A AND C—F

1. Morphology and chromosome numbers

MÜNTZING (1928, Tab. 6) tried to hybridize 7 different biotypes of *Potentilla collina* as the female parent to a series of different biotypes of the related species *Tabernaemontani*, *Crantzii* and *argentea*. Only maternal plants were obtained, however, and also in a few crosses between different biotypes of *collina* the result was identical.[1]

As, in any case, the chances to get true *collina* hybrids may be expected to be greater in crosses between biotypes of the same species than in crosses between different species, new crosses between the *collina* biotypes C—A and C—F were undertaken in 1945. These biotypes are conspicuously different morphologically, and especially C—F has a characteristic appearance with bright green, almost glabrous leaves. The chromosome number of C—A as well as C—F is $2n=42$.

From the cross *C—F*×*C—A* a total of 104 plants were raised, which were cultivated at the side of a progeny of pure C—F. This latter progeny, consisting of 12 plants, was quite uniform. In the cross progeny, 98 plants were purely maternal and identical with the plants in the C—F progeny. However, the remaining 6 plants were more or less clearly deviating, and some of them clearly combined morphological features of both parents. They also seemed to be triploid or tetraploid in relation to the parents. One of these plants did not flower but from the other ones five progenies could be raised; this will be discussed separately as follows:

Progeny 0109. The mother plant of this progeny was coarse and broad-leaved with a somewhat disharmonious leaf shape and was supposed to be a hybrid between C—F and C—A having 63 or 84 chromosomes. The latter alternative was the correct one judging from the offspring (after open pollination), which consisted of 14 individuals. Eleven of these plants were uniform and similar to the mother plant, three plants being more or less clearly deviating. Chromosome counts were made in 8 of the typical plants, all of these 8 plants having $2n=\pm 84$. Most probably the mother plant had arisen by fertilization of an unreduced egg cell of C—F with an unreduced male gamete of C—A. As meiosis in C—A is known to be irregular and pollen size

[1] The two apparently deviating individuals mentioned in the table were later found to be maternals like the other plants.

variable (*cf.* A. and G. MÜNTZING, 1943), a certain proportion of unreduced pollen grains are certainly formed.

Progenies 0110, 0111 and 0112 were raised from three mother plants supposed to be F_1 hybrids between C—F and C—A. They were more or less vigorous, and had a more harmonious appearance than the mother plant of the previous progeny. The thre progenies, consisting of 25, 15 and 18 plants respectively, were each uniform *inter se* but differed from each other in vigour and other respects. All the plants were cytologically examined and were found to have $2n=63$ or ± 63. Thus, from this it is clear that the mother plants had arisen from unreduced egg cells of C—F, which had been fertilized by reduced male gametes of C—A. The hybrids thus obtained had apomictic seed formation.

Progeny 0113, however, represents a deviating behaviour. Of the 28 plants in this progeny, one appeared to be a pure C—F plant, the other 27, on the contrary, being true hybrids. The C—F plant was found to have $2n=\pm 42$ and may represent and admixture. Among the hybrid plants the chromosome numbers were not uniform but showed the following variation:

Chromosome numbers:	± 63, 63 .... ± 82, ± 83, ± 84, 84
Number of individuals:	3 1 .... 3 3 7 6

Thus, there is a fairly clearcut division into two groups one with ± 63 and the other with ± 84 chromosomes. Morphologically, the mother plant did not differ from the three previous mother plants giving constant progenies with 63 chromosomes. Under such circumstances the most plausible explanation seems to be that also the mother of progeny 0113 had $2n=\pm 63$, that the egg cells were all unreduced, that some of them developed parthenogenetically but most of them after fertilization. As the progeny was raised from seeds obtained after open pollination of the hybrid mother plant, it is most likely that the male parents of the present progeny have been the maternal sister plants with 42 chromosomes (pure C—F), constituting most of the offspring of the cross C—F×C—A. Unreduced egg cells with 63 chromosomes+male gametes with ± 21 chromosomes will give hybrid plants with ± 84 chromosomes. Morphological observations, before the chromosome numbers were known, did not reveal the occurrence of two different chromosome categories in the offspring, the hybrid plants all being of similar appearance. As most of the plants had ± 84 chromosomes and only 4 plants $2n=\pm 63$, and as, moreover, both categories represent a combination of chromosomes from C—F and C—A (42 C—F+21 C—A or 63 C—F+21 C—A), this heterogeneity may easily be overlooked.

The cross *C—A*×*C—F* resulted in 76 plants which, unfortunately, were not cytologically examined. Morphological inspection revealed that most of the plants were maternal C—A plants. Only two clearly deviating individuals could be found, both of them apparently having an increased number of chromosomes. One of them gave rise to *Progeny 0115*, which consisted of 15 individuals. Twelve of these plants had 84 or ±84 chromosomes and appeared to be identical with tetraploid C—A, no trace of C—F being visible. The three remaining plants were typical reversions with $2n=42$. The most likely explanation of this case seems to be that the mother plant had arisen by parthenogenetic development of an egg cell with twice the somatic chromosome number. As it appeared in a progeny raised from a cross between C—A and C—F, we cannot here assume that the tetraploid plant came from the fusion of unreduced male and female gametes. In such a case it should certainly have shown some morphological influence of C—F.

The other deviating plant in the cross C—A×C—F gave rise to *Progeny 0116*, consisting of 17 plants. These plants represented the same type and appeared to be triploid, showing less extreme signs of polyploidy than the previous progeny. Chromosome counts are available from 5 of these plants, 4 of which were found to have $2n=\pm 65$, 1 plant $2n=\pm 63$. Thus, it is highly probable that the mother plant really had a triploid constitution with 42 chromosomes from C—A and ±21 from C—B. It is not excluded that the plants of this cross, C—A×C—B, may have included a few more triploid hybrids, as it was less easy to distinguish hybrids from maternal plants in this cross than in the reciprocal one.

At any rate, it is clear from the data given above that neither C—A nor C—F are completely apomictic, a low proportion of hybrids being formed. These hybrids, however, were all derived from fertilizations of unreduced egg cells. Most of them gave constant progenies owing to apomictic reproduction. In one case most of the unreduced embryosacs were fertilized.

2. Pollen fertility

Though limited to a total of 47 plants the data on pollen fertility in the crosses C—B×C—A and reciprocally may be briefly mentioned.

As is evident from Table 5 the average percentages of good pollen in the parent biotypes C—F and C—A were 51.7 and 25.0 respectively. The maternal plants in the cross C—F×C—A had about the same degree of pollen fertility (47.7 per cent) as the C—F parent, and the same is

TABLE 5. *Pollen fertility in the cross C—F×C—A and reciprocally.*

Category	Per cent good pollen											n	$\bar{x}$
	0	10	20	30	40	50	60	70	80	90	100		
Pure C—F					3	3	3	1	2			12	51.7
Maternal plants from C—F×C—A					4	3	1	3				11	47.7
Hybrids between C—F and C—A					2	1	—	1	1			5	51.0
Pure C—A			3	3	3							9	25.0
Maternal plants from C—A×C—F			4	2	2							8	22.5
Hybrid between C—A and C—F								1				1	(65.0)
84-chromosome C—A from C—A×C—F											1	1	(95.0)

true of the other category of maternal plants, from the cross C—A×C—F, the average value of these C—A plants being 22.5. The true C—F×C—A hybrids, most of which had ±63 chromosomes, were fairly sterile with an average of 51.0 per cent good pollen. Of the two aberrant plants from the reciprocal cross, C—A×C—F, the triploid hybrid had about 65 per cent good pollen, and the tetraploid plant, which was most probably not a hybrid but only a chromosome-doubled C—A, had between 90 and 100 per cent good pollen.

Of the two parent biotypes C—A is evidently more sterile than C—F. The difference is probably still larger than indicated in the table, two plants of C—A and two maternal plants of the cross C—A×C—F probably being male sterile with non-dehiscing anthers.

IV. THE FORMATION OF HETEROPLOID ABERRANTS IN POTENTILLA CRANTZII AND TABERNAEMONTANI

1. Data from P. Crantzii

Like *P. collina*, *P. Crantzii* is quite predominantly apomictic but not absolutely constant. In some of the progenies cultivated in the field occasional deviating individuals have been found. As a rule these aberrants have also deviating chromosome numbers, being either aneuploid or polyploid. Some cases of this kind will be described below.

The first case was observed in *Cr—C, a biotype with 2n=49 obtained from the Botanical Garden of Nantes.* In one progeny of this strain a single plant was observed to deviate considerably from the sister plants, especially by having semi-filled flowers with 10—13 petals instead of the normal 5. This aberrant also had larger dimensions, broader and thicker leaves, and coarser stems than the normal sister plants and was therefore suspected of being tetraploid or triploid in relation to the normal plants of the strain. This was not the case, however, the plant apparently having $2n=42$ and consequently 7 chromosomes *less* than the typical number of the strain. More detailed counts in progenies of this aberrant revealed that the correct chromosome number of this plant was not 42 but rather 42+1 fragment. Twice offspring was raised from the plant in question, a total number of 26 daughter plants being available for observations and chromosome counts. The progenies were quite uniform and represented the same morphology as the mother plant.

In the first progeny my technical assistant, Miss M. PALM, counted

$2n=43$ or ± 43 in 15 plants and $2n=42$ in 3 plants. In the second progeny, raised one year later, she found $2n=42+1$ f in all the 8 plants examined. At the same time a new progeny of normal Cr—C was raised, and in 4 plants examined the chromosome numbers were found to be ± 46, 47, $\pm 46+1$ f, $47+1$ f. This seems to indicate that the previous finding of 49 chromosomes in Cr—C may be slightly wrong, but the evidence available on this point is not quite conclusive. At any rate, it is clear that the aberrant individual in question had lost several chromosomes, which means a change from exact or approximate heptaploidy to hyperhexaploidy. It is almost euploid, but the presence of an additional small chromosome or fragment implies that it should be classified as aneuploid.

A more detailed study of aneuploid aberrants was carried out with regard to *a Crantzii biotype from Fossli, Norway*. The first progeny of this biotype, derived from a single mother plant, was raised in 1940 and consisted of 31 plants. The progeny was constant with the exception of two deviating plants, 211—7 and 211—11. Both these plants were weaker than their normal sister plants, and in 211—11 there were also definite morphological deviations (plant more gracile, stems less red-coloured than in the sister plants, leaves smaller but with longer hairs, flowers and anthers smaller, petals narrower etc.). From 211—11 a progeny after open pollination was raised. This progeny (1943—423) consisted of 42 plants, which were all alike, of the same type as the mother plant and clearly differing from a progeny of normal *Crantzii* Fossli, raised at the same time (1943—421). Chromosome counts were made in 11 plants of 1943—423, the result being $2n=42$ in 9 plants and ± 42 in 2 plants. The normal sister progeny, 1943—421, consisted of 33 plants, which were all alike. Chromosome counts were made in 5 of these plants, which were all found to have $2n=42$, a number already previously found in this biotype.

Thus, it is clear that the new biotype, represented by the deviating plant 211—11 and its offspring, had the same chromosome number as the original biotype. Most probably it had arisen by an occasional fertilization of a reduced embryosac. Although there is reason to assume that meiosis in *Crantzii* Fossli may be irregular (*cf.* MÜNTZING, 1928, p. 278), sexual reproduction must not necessarily lead to a changed chromosome number. This, however, must have happened at the formation of the other deviating plant, 211—7. A progeny of this plant was raised in 1946 and consisted of 20 plants. Some of these plants were weaker than the sister plants, but otherwise this progeny (1946—

48) was constant and clearly different from sister progenies of normal *Crantzii* Fossli. Chromosome counts were made in 18 of the plants with the following result:

Chromosome number:	24	±41,	±42,	±43,	43
Number of plants:	1	1	1	5	5

Evidently, $2n=43$ is the typical chromosome number of this progeny and probably also occurred in the deviating mother plant. It cannot be stated with certainty that the plants wih ±41 and ±42 really have numbers lower than 43.

The occurrence of a plant with $2n=24$ is highly interesting, as this must represent a case of parthenogenetic development of a reduced egg cell. The deviation from the exactly reduced number, 21, may be due to meiotic irregularities, such disturbances being observed in other biotypes of *Crantzii* and *Tabernaemontani* (MÜNTZING, 1928, 1931). The assumption that meiotic irregularities also occur in *Crantzii* Fossli is supported by the fact that this biotype just as other biotypes of *Crantzii* and *Tabernaemontani* has a poor pollen fertility (*cf.* below p. 312). As the chromosome number 24 is unusual and unexpected, a new slide of the same fixation was studied, but again the result was $2n=24$. Unfortunately, the plant in question had died long before the chromosome numbers of this material were determined, and no detailed notes concerning its appearance are available. Evidently it did not differ conspicuously from the sister plants.

This aberrant biotype was studied one generation more by raising progenies from one plant with $2n=43$ (48—1) and another plant (48—2) with $2n=\pm 43$. From the first of these two individuals a small progeny of eight weak plants was obtained, in which the following chromosome numbers were counted: 43 (2), ±43 (3), ±42 (2), ±80 (1). Thus, most of the plants had 43 chromosomes, but one individual had got its chromosome number doubled. Only three daughter plants of 48—2 could be obtained, these plants also being quite weak. Their chromosome numbers were found to be 43 (2) and 42 (1).

Before leaving the biotype *Crantzii* Fossli, it should be mentioned that new progenies were raised from three other plants appearing in the offspring of normal *Crantzii* Fossli and suspected of being deviating. However, in all three cases the progenies were quite normal, showing that the deviations in the mother plants had only been caused by environmental disturbances. Altogether these progenies comprised a total of 37 plants, 12 of which were cytologically examined with the following

result: $2n=42$ (10), ± 42 (1), ± 43 (1). In another progeny from a quite normal plant of the same biotype, consisting of 20 individuals, one plant was probably aberrant though this has not been further studied. Two normal sister plants had $2n=42$.

At any rate, the data presented above demonstrate that occasionally fertilization of reduced embryosacs may occur in *Crantzii* Fossli, the result being new recombined biotypes. Some of them are aneuploid, others have retained the hexaploid chromosome number of the mother biotype. In rare cases reduced embryosacs may develop parthenogenetically and give rise to polyhaploid aberrants. One of the aneuploid aberrants gave rise to a chromosome-doubled daughter plant.

A third case of formation of a probably aneuploid aberrant was met with in the biotype *Crantzii, Pallemjokk*, collected in the Abisko area in North Sweden. The first progeny consisted of 11 plants, one of which was deviating. This plant was weaker than the sister plants and had somewhat shrivelled leaves. Its chromosome number was not determined but judging from its progeny it may have been $2n=43$. The offspring (1946—54) of this aberrant plant only consisted of five plants, which were on an average weaker than plants derived from normal individuals of the biotype cultivated at the same time. Among the five plants of 1946—54 there was an obvious degree of variation in vigour and probably also in chromosome number, which may be specified as follows:

Plant No.	Vigour	Chromosome counts [1]
1	Very weak	42, 42
2	Weak	± 43, ± 43
3	Fairly vigorous	± 43, ± 43
4	» »	43, ± 43
5	Strongly aberrant	± 41

[1] Each value based on counts of ± 3 plants.

From these data it seems probable that the mother plant and three of the daughter plants had $2n=43$ and represent the same aneuploid biotype. Plants 1 and 5 may be secondary recombination products of this biotype with slightly lower chromosome number and still poorer vigour.

Normal, vigorous plants of the biotype have $2n=42$, as was controlled in altogether 7 plants from three different progenies, composed of 47 plants in all. Of these plants 46 were normal and uniform, the only deviating plant being the one described above.

Aneuploid and polyploid aberrants were also observed in progenies

of *a Crantzii biotype from Bondkyrka*, Uppland, Middle Sweden. The original progeny (1943—457) consisted of 7 plants, one of which (457—1) clearly deviated in leaf colour and by having somewhat larger flowers. Three of the normal plants were found to have $2n=42$. In the next generation an offspring of 8 plants were obtained from 457—1. They were uniform and similar to a progeny of 6 plants raised from a normal sister plant (457—5) of 457—1. However, between the two progenies there was a certain degree of morphological difference and also a difference in chromosome number. In the progeny derived from 457—1, chromosome counts could be made in six plants, which were all found to have $2n=43$ (5) or ± 43 (1). In the other progeny, consisting of 6 plants, 5 individuals were typical, two of them being controlled to have $2n=42$. The sixth plant in this progeny appeared to be a polyploid aberrant, and this was indeed verified by raising a new progeny from this individual. This progeny, 1954—011, consisted of 6 individuals, which were found to have the following chromosome numbers: 84 (3), ± 84 (1), 80 (1) and 42 (1). Most probably the aberrant mother plant had $2n=84$ like most of the offspring. The plant with 80 chromosomes may have arisen by fertilization of a reduced embryosac and the plant with $2n=42$ is a reversion plant of the same kind as those previously met with in *Potentilla collina* (*cf.* above p. 281).

The difference between the euploid typical biotype with $2n=42$ and the deviating aneuploid with $2n=43$, described above, was studied one more generation. The former type was represented by 16 uniform plants, two of which were cytologically examined and found to have $2n=42$. The deviating aneuploid was represented by 20 apparently uniform plants, which were weaker and had thinner leaves than the typical sister progeny. Chromosome counts were made in 10 of these 20 plants, the chromosome number 43 being found in 9 plants, and ± 43 in one plant. Thus, it is quite certain that in this strain of *P. Crantzii*, aneuploid as well as polyploid aberrants may sometimes be formed, the polyploids having the same ability as in *P. collina* to revert to $\pm$ the original chromosome number.

Though fragmentary, some observations on *pollen fertility in the aberrants* may be mentioned. Thus, in 1945, pollen samples were not only taken from normal *Crantzii* Fossli but also from the deviating type with unchanged chromosome number. In the original biotype the average percentage of good pollen was only 15.0 (Table 6) whereas the corresponding value in the deviating type ranged from 70 to 90 per cent with an average of 78.3. Thus, the presumed genetic recombination

(*cf.* above p. 306) has not only resulted in a somewhat deviating morphological appearance but also in a considerably improved pollen fertility. Data from 1948 demonstrate that also the hyperhexaploid aberrant biotype with $2n=43$ in *Crantzii* Fossli had got its pollen fertility improved. A progeny of six individuals, belonging to this biotype, had a range from 50 to 70 and an average of 57.0 per cent good pollen as compared to a range from 20 to 50 and an average of 32.8 in the original biotype (18 plants examined).

In a *Crantzii* biotype from Bondkyrka, on the other hand, the 43-chromosome aberrant biotype had slightly lower pollen fertility than normal sister plants with $2n=42$. The same thing was repeated in *Crantzii* Pallemjokk, in which the aberrants with $2n=43$ had only about 60 per cent good pollen as compared to 89 per cent in the normal, original biotype. All this probably indicates that in biotypes with poor pollen, recombination generally leads to improved fertility, the reverse being true if the original biotype has a good pollen fertility.

2. Data from P. Tabernaemontani

As a rule progenies of single plants of *P. Tabernaemontani* are perfectly constant and uniform but in a few cases deviating plants have been observed; this demonstrates that the apomictic seed formation in this species, just as in *P. Crantzii*, is not entirely without exceptions.

Thus, for instance, in 1942, a biotype of *Tabernaemontani* from Bäckaskog, Scania, was represented by a progeny of 29 plants, 28 of which were typical and 1 clearly deviating, showing signs of chromosome doubling. In 1944, a progeny consisting of 16 plants was raised from this deviating individual. This progeny appeared to be constant and was composed of vigorous plants with large and broad leaflets. The constancy as well as the supposed increase in the chromosome number was verified by chromosome counts in 15 of the plants, the following numbers being found: ± 77 (4), ± 78 (7), 78 (2), ± 79 (2). Most probably 78 is the correct number in all the plants. Normal plants of the original biotype have $2n=49$ [counts in 3 plants giving the result 49 (2), ± 50 (1)]. Thus, the original aberrant and its apomictic offspring are approximately triploid in relation to the mother biotype. Just as in a similar case in *P. collina* (*cf.* above, p. 284) the triploid must have arisen after fertilization of an unreduced egg cell with 49 chromosomes by a reduced male gamete with 29 chromosomes.

In some other biotypes of *P. Tabernaemontani*, aberrants have also been observed, these deviating plants probably being polyploid or an-

euploid though this has not been definitely proved. However, the single clear case described above is sufficient to demonstrate that *P. Crantzii* and *Tabernaemontani* are essentially similar with regard to the degree of constancy.

V. CYTOTYPES AND POLYMORPHISM IN P. CRANTZII AND TABERNAEMONTANI

1. Data from P. Crantzii

Altogether the chromosome numbers have been determined in about 25 different biotypes of the species, most of them being hexaploid ($2n=42$). In two cases the biotypes were heptaploid, having $2n=49$, and in one case the number was aneuploid, though approximately heptaploid ($2n=48$). In a few cases progenies from the same or adjoining localities were found to represent the same biotype, judging from morphological identity; otherwise they were always different, thus representing a pronounced polymorphism. Within each progeny there was as a rule complete constancy, but in a few cases apparently aberrant individuals were observed to occur.

The following information about the geographic origin of the various biotypes may be given:

Hexaploid biotypes ($2n=42$). In the province of *Uppland*: Old Upsala (Björkeby and Flogsta), Börje (Broby), Bondkyrka (Kvarnbo and Hällby). In *Dalarna*: Folkärna, Hedemora, Rättvik. In *Gästrikland*: Hamrånge (Axmar). In *Jämtland*: Ulriksfors. In *Lappland*: Saxnäs, Kvikkjokk, Pallemjokk, Låktatjocko and Nuolja. In *Norway:* Fossli. Two biotypes previously described (MÜNTZING, 1928, 1931), Cr—A from Västergötland and Cr—B from the Botanical Garden of Helsingfors, also belong to the hexaploid category.

Heptaploid biotypes ($2n=49$). In the province of Uppland: Old Upsala (Björkeby). Cr—C, previously described and also discussed above (p. 305), a biotype from the Botan. Garden of Nantes.

Aneuploid biotype ($2n=48$). In Dalarne: Rättvik.

In most cases the chromosome counts were made in three individuals per progeny. The aneuploid biotype with $2n=48$ was studied more closely. The original progeny only contained 4 plants, 2 of which were examined and found to have $2n=48$. A new progeny, consisting of 10 plants, was raised and found to be uniform. Chromosome counts could be made in 9 of these plants with the following result: $2n=48$ (1), ± 48 (5), ± 49 (2), ± 47 (1). Though not absolutely conclusive, these data, in combination with those from the previous generation, strongly

TABLE 6. *Pollen fertility in biotypes of P. Crantzii.*

Biotype	Per cent good pollen											n	x̄
	0	10	20	30	40	50	60	70	80	90	100		
Norway, Fossli	2	6	2									10	15.0
Uppl., Old Upsala (2n=49)								4	4	2		10	73.0
» , » » (2=42)			4	4	1							9	21.7
» , Börje and Bondkyrka					3	9	6					18	46.7
» , Bondkyrka (Kvarnbo)								3				3	(65.0)
» , Bondkyrka and Old Upsala							6	4				10	59.0
» , Bodkyrka (Flogsta) a			1	7	6	1						15	29.7
» , » » b						1	4	5				10	59.0
Dalarna, Folkärna						6	4	1				11	50.5
» , Hedemora						6	2					8	47.5
» , Rättvik a (2n=42)				2	6	2						10	35.0
» , » b (2n=48)							1	3				4	(62.5)
» , » b (2n=42)					1	3	2					6	46.7
» , » b (2n=42)								2	3			5	71.0
Gästrikland, Hamrånge						5	4					9	49.4
Lappland, Låktatjokko								1	3			4	(72.5)
» , Nuolja										5	5	10	90.0
» , Pallemjokk										6	4	10	89.0
» , Kvikkjokk									3	6		9	81.7
» , Saxnäs							1	—	1	1		3	(71.7)
Jämtland, Ulriksfors						7	2	1				10	49.0

indicate that the correct number is $2n=48$, occasional aneuploid biotypes thus occurring in nature.

Pollen fertility in *P. Crantzii*, just as in *P. collina* and in polyploid biotypes of *P. argentea*, was found to be highly variable and on an average markedly reduced. However, as is evident from Table 6, based on pollen counts made in 1945, there are very pronounced differences between biotypes; these differences cover the whole range from 15 per cent good pollen in *Crantzii* Fossli to 90 per cent good pollen in the biotype from Nuolja. No statistical treatment is needed to prove the significance of this heterogeneity.

Before leaving this table, it should be mentioned that when more than one name is given for a biotype, this means that the progenies from both localities were found to be identical. This was the case with material from Börje and Bondkyrka, Uppland, and with another biotype from Bondkyrka which was identical with material from Old Upsala. The denominations a and b after Bondkyrka (Flogsta) and Rättvik indicate different sublocalities at these places. Thus Rättvik, locality a, is represented by one hexaploid biotype, locality b by two different hexaploids and the subheptaploid biotype having 48 chromosomes. In all biotypes for which the chromosome number is not given, $2n=42$ is understood.

2. Data from P. Tabernaemontani

Up to the present a total of 18 different apomictic biotypes of *P. Tabernaemontani* have been in culture and have been cytologically examined. The most frequent chromosome number is $2n=42$, which was found in biotypes from the following localities: In the island of Öland: Böda (northern point of the island), Vickely (2 different biotypes) and Resmo; in Uppland: Bondkyrka (3 different biotypes).

Several biotypes have been found to be heptaploid with $2n=49$. This was true of 3 different biotypes from Bondkyrka in the province of Uppland, and also another biotype from Uppland (Danmarks sn, Berga) was found to have the same number. The heptaploids, however, also occur in other Swedish provinces, $2n=49$ being found in a biotype from Bäckaskog, Scania.

The next multiple of seven, $2n=56$, has been observed in a single biotype from Ottenby, in the island of Öland. From 56 there is a gap until $2n=84$, which has previously been found in another biotype from Öland, the exact locality not being known (MÜNTZING, 1928).

At least one biotype is aneuploid, having $2n=50$. This type belonged

to the material from Bondkyrka, Uppland, from which region biotypes with 42 and 49 chromosomes have already been mentioned. The first progeny with $2n=50$ was from Bondkyrka (Flogsta) and consisted of 10 identical plants, three of which were found to have $2n=50$. A new progeny was raised, consisting of 8 plants and showing the same constancy. Chromosome counts in 7 plants gave the following result: $2n=50$ (5) and ± 50 (2). Thus, the aneuploidy of this biotype is well established. Morphologically this type is similar to another biotype with $2n=49$ from the same local region. However, there is a clear difference, the 50-chromosome type having smaller leaves and a more gracile appearance than the 49-chromosome type.

Another biotype from the Bondkyrka region (Kvarnbo) is probably also aneuploid but with $2n=\pm 60$. The original progeny consisted of only 2 similar plants determined to have 60 and ± 60 chromosomes respectively. A new constant progeny of 8 plants was raised, 2 of which were cytologically examined and found to have ± 58 and ± 60 chromosomes respectively. This certainly indicates a chromosome number intermediate between 56 and 63. Morphologically, this biotype was very similar to, or even identical with, a biotype from Bondkyrka (Flogsta). In this biotype, represented by 9 plants, only one plant could be cytologically examined, the result being ± 63. The data available are not sufficient to decide whether this apparent difference in chromosome number is really significant.

Finally, it should be mentioned that *pollen fertility* in *P. Tabernaemontani*, just as in *Crantzii*, has been observed to be more or less reduced in various biotypes, but the data now available are too meagre to justify a detailed presentation. Some information on pollen fertility in the *Tabernaemontani*-biotypes T—A and T—B and their constant hybrid was given previously (A. and G. MÜNTZING, 1941, p. 269).

3. An apomictic hybrid between Crantzii and Tabernaemontani

As we have seen above, several biotypes of *Crantzii* and *Tabernaemontani* were derived from the province of Uppland, where they had been collected by Doctors J. A. NANNFELDT and G. LOHAMMAR and kindly put at my disposal for cytological examination and field cultures. Though the collector in some cases had hesitated as to the correct classification of the plants growing in natural habitats, almost all these biotypes, when cultivated in the experimental field, very clearly fell into the two categories *Crantzii* and *Tabernaemontani*. There was one exception, however, a biotype that certainly represented a hybrid be-

tween the two species. This biotype was first represented by two, evidently identical plants (6282 pl. 1 and 2) which before flowering were classified as *Tabernaemontani* on account of their loose and spreading shoot system. The flower, however, turned out to be large and to have the orange spot at the base of the petals, which is characteristic of *P. Crantzii*. Pollen fertility in the hybrids was good, 70 per cent of the pollen grains being apparently normal.

By raising new progenies from both the original plants, the hybrid nature of this material became quite clear. However, both these progenies, each consisting of 12 plants, were quite true-breeding and showed no trace of segregation. The chromosome number in both mother plants was found to be $2n=62$. In each of the progenies, 3 plants were cytologically examined, the total result being $2n=62$ in 5 plants and ± 61 in 1 plant. As the latter value probably does not represent a true deviation, we have here a true-breeding biotype combining morphological characters of both the supposed parent species. Considering that most biotypes of *Crantzii* and *Tabernaemontani* are hexaploid with $2n=42$, a plausible hypothesis is that the biotype in question has arisen by an exceptional fertilization of an unreduced egg cell of one of the species by a reduced male gamete of the other species. The data available are not sufficient to decide whether *Tabernaemontani* or *Crantzii* has been the female parent of the cross. At any rate, the hybrid thus obtained has retained the ability of apomictic seed formation prevailing in both parent species.

VI. DISCUSSION

1. »Tetraploids» and reversions in Potentilla collina

In *P. collina* most plants arise by parthenogenetic development of unreduced egg cells and are therefore identical with their mothers. Only in rare cases have aberrants with changed chromosome numbers been observed to occur. Thus, in the best studied biotype, C—A, a total of 249 individuals have been under observation during the course of years, and of these 245 were typical and maternal and only 4 deviating aberrants. As reported above two of these plants were (poly-) tetraploid with $2n=84$, the other two $\pm$triploid. Single tetraploid aberrants have also been observed in two other biotypes of *collina*, and a single triploid plant with $2n=63$ was observed in the *collina* biotype C—F among a total of 64 normal sister plants.

In *Taraxacum* (SØRENSEN and GUDJONSSON, 1946) gigas aberrants

with doubled chromosome number frequently arise as chimaeras, for which reason it is assumed that the polyploid constitution is initiated by an abnormal mitosis in the vegetative tissues. This mode of formation probably does not occur in *Potentilla*, no trace of a chimaerical constitution being seen in the tetraploids obtained.

With regard to their mode of reproduction the tetraploids are essentially similar to the original biotypes, most embryos arising by parthenogenetic development of unreduced ovules. This is evident from crossing experiments, giving maternal offspring just as seeds from open pollination. However, a striking feature of the tetraploids is that a considerable proportion of their offspring is represented by reversion plants having approximately half the chromosome number of the mother plant and consequently the same, or approximately the same chromosome number as the original biotype. The production of such plants shows that some of the embryosacs of the 84-chromosome mother plant are reduced, and that the egg cells in such embryosacs may develop into embryos parthenogenetically. HÅKANSSON (1946), who has studied this *Potentilla* material embryologically, actually observed the occurrence of reduced embryosacs in »*collina* gigas». He also made the observation that such embryosacs are not present either in normal C—A or in the reversion plants.

Thus, normal C—A may be said to be more strictly apomictic than gigas C—A, in which a fairly large proportion of the embryosacs are reduced. The frequency of such embryosacs may to some extent be inferred from the frequency of reversion plants. All data available concerning the frequency of such plants in *P. collina* may be summarized as follows:

Number and kinds of plants in progenies of tetraploids.

Progenies	Maternals	Reversion plants
84-chromosome C—A, 1st strain	78	15
» » , 2nd »	9	5
» *collina*, Resmo	9	1
» » , Vickleby (?)	5	2
» C—A from C—A×C—F	12	3
Total	113	26

According to the total data the frequency of reversion plants is as high as *18.7 per cent.*

Owing to slight meiotic irregularities in the gigas plants with 84 chromosomes the chromosome numbers of the reversion plants are not

TABLE 7. *Numbers and kinds of plants in progenies of reversion plants.*

Progenies	Maternals	Tetraploids	± Triploids	Other aberrants
1944—518 (2n=40), 3 progenies, open pollination	77	13	1	
» —526 (2n=39), 2 » , » »	58	16		
» —520 (2n=±42), open pollination	47	7		4[1]
» —521 (2n=42), isolation	18			
» —522 » , open pollination	45	7	1?	
» —523 » , isolation	40			
» —524 » , open pollination	45	4		
» —527 » , isolation	18	1		
» —528 » , open pollination	35	3		
336—28 (2n=39)×C—A (2n=42)	49	3		
» —40 (2n=42)×C—F (2n=42)	50			
Three reversion plants (2n=42)×tetr. C—A (2n=84)	44	4		
Total	526	58	1+1?	4

[1] Of these four plants one had $2n=41$, the others were morphologically deviating but not cytologically examined.

always 42 but have been observed to range from 39 to 43. Fifteen progenies, comprising a total of 590 individuals, have been raised from reversion plants, chiefly after open pollination, in some cases after isolation. In the first place these progenies demonstrate that the reversion plants are predominantly apomictic, the great majority of the plants being maternal. However, about 10 per cent of the plants may be called secondary tetraploids, which have got their chromosome number doubled. In a few cases also triploids or other aberrants have been observed to occur. The distribution of these categories in the families was found to be the following (Tab. 7):

The frequency of tetraploids seems to be different in different families. In three cases progenies were raised after isolation as well as after open pollination of the mother plants. In the former cases a total of 76 maternals and 1 single tetraploid were obtained, whereas the same mother plants after open pollination gave a total of 137 maternals and 18 tetraploids. The heterogeneity of these ratios was tested with the χ^2 method, which gives a χ^2 of 7.28, corresponding to a P smaller than 0.01. Thus, the difference is significant. The most plausible explanation of this fact seems to be that some of the tetraploids have arisen from spontaneous crosses with plants of tetraploid C—A ($2n=84$). As the reversion plants were growing in a progeny of tetraploid C—A and were

surrounded by a majority of tetraploid plants which, moreover, have much better pollen fertility than normal C—A and the reversions, such spontaneous crosses may easily have taken place. Consequently, we may conclude that part of the secondary tetraploids have arisen in this way by fertilization of unreduced egg cells with ± 42 chromosomes by pollen, having the same chromosome number.

However, this assumption is rendered difficult by the fact that the tetraploids in the aneuploid progenies 1944—518 and —526 cannot have arisen in this way. As already pointed out above, the chromosome numbers of the tetraploids in these progenies are such that spontaneous crosses with tetraploid C—A are excluded, the tetraploids in these cases having arisen either by fertilization of unreduced egg cells with unreduced pollen grains of the same plant, by parthenogenetic development of unreduced egg cells followed by somatic chromosome doubling or by parthenogenetic development of egg cells having twice the somatic chromosome number. The single tetraploid in progeny 1944—527, raised after isolation, must also have arisen in one of these ways and not as a result of a spontaneous cross.

Thus, the results obtained strongly indicate that the tetraploids in different progenies have arisen in different ways. The reason for this are not all clear, but the differences may for instance depend on various frequencies of unreduced pollen grains in different reversion plants or on various frequencies of embryosacs with twice the somatic chromosome number.

On an average, the frequency of tetraploids in the offspring of reversion plants is much higher than the frequency of tetraploids in the offspring of normal C—A. As pointed out above, only 2 tetraploids were obtained among a total of 249 plants, which is less than 1 per cent. In the offspring of the reversion plants about 10 per cent of the plants were tetraploid, which certainly indicates a lower degree of stability than in the original strain. In the most aneuploid biotype, having $2n=39$, the corresponding frequency was even as high as 22 per cent.

The most interesting feature of the reversion biotypes is their genotypical diversity. This is rather self-evident with regard to the aneuploid reversion types, but with the help of various measurements (pp. 294—298) it could be demonstrated that also reversion types having $2n=42$, the same chromosome number as the original biotype, may be different *inter se*. Consequently, some of them, at least, also differ from normal C—A. As these differences, at least in some cases, do not involve any inferiority in vigour, we may conclude that *by spontaneous chromo-*

some doubling and reversion new biotypes of P. collina are continuously being formed in nature. It is highly probably that this process is a powerful means of increasing the polymorphism of the species.

As previously reported (A. and G. MÜNTZING, 1943), meiosis in normal C—A ($2n=42$) is markedly irregular, the average number of univalents at I—M being as high as 8.10. In contrast to this, meiosis in C—A gigas, having $2n=84$, is very regular, the great majority of the chromosomes forming bivalents at I—M, and the number of univalents present being quite low (0.96 per cell). Thus, meiosis in normal C—A indicates a hybrid constitution, and the product with doubled chromosome number has the regular meiosis characteristic of allopolyploids. If meiosis in the »tetraploid» were perfectly regular, only one kind of gametes would be formed, and all reversion plant, representing the parthenogenetic development of reduced gametes, ought to be isogenic. As this has definitely been found not to be the case, we must conclude that bivalents in the 84-chromosome type are not always formed by the same chromosomes. As there are not only univalents but also many bivalents at meiosis in normal C—A, multivalents might be expected in the chromosome-doubled C—A. As such configurations have not been observed to occur but only bivalents and a low frequency of univalents, this may either mean that there is a strong degree of differential affinity or a random formation of bivalents between more or less homologous chromosomes. As there is a genotypical diversity between the vigorous reversion plants with 42 chromosomes, the latter alternative must be the correct one. Consequently, the mode of meiosis in the chromosome-doubled C—A resembles that in Scandinavian strains of *Phleum pratense*, in which the groups of 6 homologous or partially homologous chromosomes do not form multiple associations but unite at random to form three bivalents (NORDENSKIÖLD, 1953, 1957).

2. »Triploid» aberrants

In *Potentilla collina* not only tetraploids may arise spontaneously, but also triploids may be formed now and then. This has happened twice in strain C—A, once in C—F, and once in a progeny of a reversion plant. Triploids could also be produced artificially by crosses between C—F and C—A, 4 such plants being identified. Finally, a single triploid individual was also observed to occur in a certain strain of the related species *P. Tabernaemontani*. As usual, the term »triploid» is used here to indicate plants which are triploid in relation to the original strains, these strains being already hexaploid or heptaploid. In all cases the

TABLE 8. *Number and kinds of plants in progenies of triploids.*

Mother plant	2n of mother strain	Maternal offspring	Fertilization of unreduced egg cells	Recombination products
Tripl. plant in reversion prog. 1944—518 (2n=61)	42	39		1 (2n=±40)
C—B, 535 e—11, new triploid (2n=65)	»	15		1 (2n=70 to 74)
» , 17—19, » » (2n=70)	»	12		
C—F, 74—6, » » (2n=63)	»	75		3 (1 pl. cyt. exam., 2n=±57)
From C—F×C—A, mother of 0110 (2n=63)	»	25		
» » , » » 0111 »	»	15		
» » , » » 0112 »	»	8		
» » , » » 0113 »	»	4	19	
From C—A×C—F, » » 0116 (2n=±65)	»	17		
Tripl. plant in Tabernaemontani, Bäckaskog, 2n=78	49	16		
Total		226	19	5

»triploid» deviating and fairly vigorous plants must have arisen as a result of exceptional fertilizations of unreduced egg cells, which otherwise develop parthenogenetically.

From all triploids progenies were raised, the characteristic features of which may be summarized in the following way (Table 8):

Progeny 0113 of the cross combination C—F×C—A was exceptional, most of the plants being the result of fertilization of unreduced egg cells. In all other progenies the purely maternal plants were quite dominating, and in six of them the constancy was complete, only maternal plants being obtained. In the four other progenies there were a few products of recombination, representing unions between reduced female and male gametes.

A characteristic feature of the tetraploid aberrants is the high frequency of ±diploid reversion plants in their offspring (p. 316). The most striking feature of the progenies of triploid aberrants is the total absence of such plants. The reason for this may either be non-formation of reduced embryosacs, inability of such embryosacs to develop parthenogenetically, or inviability of the reversion plants. To some extent the latter factor might be of importance, as the triploid mother plants have chromosome numbers ranging from 61 to 78, which would give reversion plants with $2n$ values ranging from about 30 to 40. However, viable aneuploids with such numbers have been obtained in other groups of *Potentilla* material (*cf.* A. and G. MÜNTZING, 1941, 1945 and MÜNTZING, 1958 b). Such aneuploids have a more or less reduced degree of vigour, it is true, but their total absence in the progenies now under discussion strongly indicates that reversion embryos are not formed at all.

HÅKANSSON (1946) has shown that in normal C—A with $2n=42$, there are no reduced embryosacs and no haploid embryos. Neither have any haploid reversion plants with $2n=21$ been obtained in progenies of normal C—A. In contrast to this C—A aberrants with $2n=84$ produce many reduced embryosacs, in which the egg cell is able to develop by parthenogenesis. Unfortunately no embryological investigations in triploid aberrants have been carried out, for these would have elucidated whether reduced egg cells are formed but are not able to develop parthenogenetically, or whether indeed such reduced egg cells are formed at all.

At any rate, there is a clear difference in this respect between triploids and tetraploids, this difference probably being caused by the difference in absolute chromosome number. This recalls the situation in hybrids

between sexual and apomictic plants of *Poa alpina*, in which some of the hybrids resulted from the union of reduced gametes of both parents, others from the fertilization of unreduced egg cells of the sexual parent with reduced pollen grains of the apomictic parent (MÜNTZING, 1940). In hybrids of the latter kind a fairly high proportion of reduced egg cells developed parthenogenetically and gave rise to haploids in the next generation. In the offspring of normal hybrids with lower chromosome numbers no such haploids appeared. HÅKANSSON (1943, 1944), who studied the embryology of this material, found that the reduced egg cells in the normal hybrids lacked the ability of parthenogenetic development, in spite of the fact that such egg cells should carry more genes for apomictic seed formation than the hybrids resulting from fertilization of unreduced ovules of the sexual parent. In these hybrids there must be relatively more genes for sexuality, but in spite of that, many of the egg cells develop parthenogenetically. The differential behaviour of the two kinds of hybrid can only be ascribed to the difference in chromosome number, a higher number increasing the tendency to parthenogenetic development (*cf.* also GUSTAFSSON, 1947, p. 73).

A difference between this case in *Poa* and the present one in *Potentilla* is that the absence of haploids in the offspring of *Potentilla collina* plants with the normal, low number ($2n=42$) is due to the absence of reduced embryosacs (HÅKANSSON, 1946), and thus it is not known whether reduced egg cells, if they had been formed in these plants, would have developed parthenogenetically. The 84-chromosome plants may be said to be *more* sexual than the normal strain, as they are capable of forming a rather high proportion of reduced embryosacs. But the apomictic tendency, in most cases, is strong enough to cause the egg cells in these embryosacs to develop parthenogenetically. In the »triploids» with ± 63 chromosomes, reduced embryosacs are not formed or their egg cells are incapable of parthenogenetic development.

3. Hybrids between different biotypes of P. collina

Normal strains of *Potentilla collina* reveal their strong degree of agamospermy not only by great constancy in the offspring but also by the fact that artificial crosses generally give purely maternal plants. However, in crosses between the *collina* biotypes C—A and C—F, some true hybrids were obtained, but in all cases these hybrids had been produced by fertilizations of unreduced egg cells. Thus, they are quite analogous to the *verna*×*verna* hybrids with ± 63 chromosomes, produced by RUTISHAUSER (1943 a, b), which also gave uniform progenies

owing to apomictic seed formation. The same result was obtained with regard to the species cross *P. canescens*×*argentea* (RUTISHAUSER, 1946), whereas the triploid hybrids between *P. argentea* and *verna* were found to be partially sexual, part of the unreduced egg cells being unable to develop parthenogenetically (RUTISHAUSER, 1945, 1948).

In my own material unreduced ovules were not only fertilized in crosses between different biotypes of *P. collina* but also in crosses between normal *collina* and its tetraploid derivative. The frequency of such fertilizations seems to be fairly high, about 15 % of the offspring of the cross normal C—A×tetraploid C—A being tetraploid. Similar results were also obtained where reversion plants of C—A were used as the female parents in crosses with tetraploid C—A.

4. Pollen fertility

It was shown above that in the *Potentilla* material under discussion pollen fertility is strongly correlated with chromosome number, types with lower numbers being more sterile than »triploid» and »tetraploid» derivatives. This correlation has probably two causes, of which the first and most important is the fact that lethal effects on the pollen grains of aneuploidy are less marked when the absolute chromosome number is high and all genes necessary for the life of the pollen grain are already reduplicated several times. The same correlation has been observed in many other groups of material and has been studied by the present writer in previous material of *Potentilla* (A. and G. MÜNTZING, 1945; MÜNTZING, 1958 b) as well as in *Dactylis* (MÜNTZING, 1937) and *Poa* (MÜNTZING, 1940, 1954).

In the present material of *Potentilla* there may also be another cause of increased pollen fertility in the polyploid derivatives. In at least some spontaneous *collina*-biotypes poor pollen fertility is correlated with an irregular meiosis, indicating a hybrid constitution with lack of homology between the genomes. Chromosome doubling in such a material may lead to an allopolyploid constitution, regular meiosis and good pollen fertility. Such were, at any rate, the conditions in the *collina* biotype C—A and its chromosome-doubled derivative (A. and G. MÜNTZING, 1943).

5. Cytotypes in P. collina

In *Potentilla collina* the few aberrants which were observed to arise spontaneously were triploid or tetraploid in relation to the mother strain. Among the reversion types obtained in the offspring of the tetra-

ploids a few were aneuploid. Hence, polyploid as well as aneuploid aberrants of *P. collina* may arise in nature. The aneuploids are probably not able to compete with euploid natural biotypes and euploid reversion types, and among the polyploids only the »triploids» would appear to have a chance to survive in nature. The »tetraploids», though showing certain gigas properties, are decidedly less vigorous than the original material.

A thorough inventary of the chromosome numbers occurring in *P. collina* in nature would be of interest. Pending such detailed information, it may be mentioned that of nine Swedish *collina* types, collected in the island of Öland, seven were found to have $2n=42$, one to have $2n=\pm 56$ and one ± 62. The biotype C—A, which is also hexaploid with $2n=42$, is obtained from the island of Gotland, Sweden. Two *collina*-types, C—B and C—G, emanating from the botanical gardens of Lyons and Uppsala, were found to be pentaploid ($2n=35$). A form of *P. collina*, called *P. praecox*, belongs to RUTISHAUSER's material and was found by him to have $2n=42$ (RUTISHAUSER, 1943).

Thus, though the observations are very limited, the hexaploid number 42 seems to be most frequent in *P. collina*, but other cytotypes also occur, having 35, ± 56 or ± 62 chromosomes. In the last-mentioned biotype (collected at Ottenby), it has not yet been possible to decide whether the correct chromosome number is 62 or 63. The observations indicate that the former number is the correct one, and if this be true, the biotype in question represents an aneuploid biotype established in nature.

6. Heteroploidy in P. Crantzii and Tabernaemontani

Aeuploidy has been observed with certainty in aberrants as well as in natural biotypes of *Potentilla Crantzii*. At least one aneuploid biotype of *P. Tabernaemontani* was found in nature, and some aberrants in the field cultures were probably also aneuploid. *P. Crantzii* and *Tabernaemontani*, which represent the *verna* group in Scandinavia, are as a rule well delimited but are evidently similar with regard to their variation in nature and in the experimental garden. Though predominantly apomictic, they are not perfectly constant but now and then produce aneuploid or polyploid aberrants. Genetically deviating aberrants with an unchanged chromosome number have also been observed. On an average these species are less stable than *P. collina*, and in some biotypes, at least, reduced egg cells are occasionally formed and fertilized, the result being aberrant individuals, which are generally aneuploid.

In one biotype of *P. Crantzii* these findings have been supported by embryological investigations, demonstrating the occurrence of reduced embryosacs (HÅKANSSON, 1946).

The aneuploid aberrants are generally less vigorous than the euploid mother biotypes. They are predominantly apomictic, generally giving uniform progenies, but sometimes the aberrants are partially sexual, giving variable progenies. In all these respects the *Potentilla* aberrants in question are similar to the aneuploid aberrants occasionally arising in apomictic biotypes of *Poa alpina* (MÜTZING, 1954). In this species also the normal biotypes are generally aneuploid whereas in *Potentilla* euploidy is the rule in nature, though some natural aneuploids have also been found.

Concerning the polyploid aberrants found in *P. Crantzii* and *Tabernaemontani* it should be mentioned that a triploid aberrant in the latter species was apomictic, giving a uniform triploid progeny, just as in similar cases in *P. collina*. A tetraploid aberrant of *P. Crantzii*, having $2n=84$, was interesting in two ways, firstly because it was able to produce reversion plants with 42 chromosomes, just as similar plants of *P. collina*, secondly because not all the tetraploid individuals in the offspring were strictly maternal. One plant out of five had only 80 chromosomes and had probably arisen by fertilization of a reduced egg cell. Thus, in this case not all the reduced egg cells developed parthenogenetically, some of them being fertilized.

Another very interesting change of chromosome number was observed in a secondary aberrant, arising from a primary aneuploid with $2n=43$. This secondary aberrant was »haploid», having $2n=24$, and was certainly the result of a parthenogenetic development of a reduced egg cell in the mother plant. In *P. collina* hexaploid biotypes with $2n=42$ only seem to produce unreduced embryosacs, but the occurrence of a »haploid» in *P. Crantzii* fits in with HÅKANSSON's observation of the occurrence of reduced embryosacs in one of the biotypes and with my own observations of recombined and generally aneuploid aberrants, which must also be derived from reduced but fertilized egg cells.

Chromosome counts in natural biotypes of *P. Crantzii* and *Tabernaemontani* have revealed the occurrence of intraspecific polyploidy also in both species. Hexaploids with $2n=42$ are most frequent, but just as in *P. collina* (p. 324) and *P. argentea* (*cf.* MÜNTZING, 1958) some biotypes have 49, 56 and 84 chromosomes. In addition to this we have some aneuploid biotypes, and in one case genomes of *Crantzii* and *Tabernaemontani* had evidently been combined to an allopolyploid with $2n=62$

chromosomes. In middle Europe other members of the *verna*-group, to which *Crantzii* and *Tabernaemontani* belong, are known to have chromosome numbers lower than 42. One such strain from the Tyrol is tetraploid ($2n=28$) and sexual (A. and G. MÜNTZING, 1945, p. 62).

Much detailed work remains to be done in order fully to elucidate the polymorphism of the *Potentilla* species studied. However, the results presented above indicate some main features of the heteroploidy and polypmorphism in *P. collina*, *Crantzii*, and *Tabernaemontani*.

SUMMARY

(1) Different biotypes of *Potentilla collina*, having apomictic seed formation, are highly constant but may produce occasional aberrants, which are either ± triploid or tetraploid in relation to the original biotype. The triploids arise by fertilization of unreduced egg cells, the tetraploids by parthenogenetic development of egg cells with twice the somatic chromosome number, by parthenogenetic development of unreduced egg cells, followed by somatic chromosome doubling, or by fertilization of unreduced egg cells by unreduced pollen grains.

(2) Triploid aberrants retain the ability of apomictic seed formation and give almost exclusively maternal offspring, only a few individuals representing recombinations resulting from fertilizations. Tetraploid aberrants are also predominantly apomictic, but at least 16 per cent of the offspring are reversion plants, having ± half the chromosome number of the mother plants. The reversion plants (with $2n$ values ranging from 39 to 43) arise from parthenogenetic development of reduced egg cells.

(3) By reversions new biotypes are formed, which are in part different *inter se* and which may differ from the original mother strain. By various measurements this was demonstrated to be valid not only for the aneuploid reversions but also for the euploid ones, having $2n=42$.

(4) The reversion plants are predominantly apomictic, the great majority of their offspring being maternal. However, about 10 per cent of the daughter plants were secondary tetraploids; and in a few cases triploids and other aberrants were also formed. On an average the frequency of secondary tetraploids in the offspring of reversion plants was much higher than the frequency of primary tetraploids in the original mother strain.

(5) Crosses were attempted between two apomictic biotypes of *P. collina*. In both reciprocal combinations most of the plants were

maternal, but a few hybrids were also obtained. These hybrids were all derived from fertilizations of unreduced egg cells. Most of them gave constant progenies owing to apomictic reproduction. In one case the majority of the unreduced embryosacs were fertilized.

(6) Scandinavian strains of *Potentilla Crantzii* and *Tabernaemontani* are predominantly apomictic but not quite stable, aneuploid or polyploid aberrants being formed in a low frequency. As in *P. collina*, tetraploid aberrants may give reversion plants with ±half the chromosome number of the mother plant. One aneuploid with 43 chromosomes instead of 42 produced a haploid with $2n=24$.

(7) Chromosome counts in natural biotypes of *P. collina*, *Crantzii* and *Tabernaemontani* have revealed the occurrence of intraspecific polyploidy in each species. Hexaploids with $2n=42$ are most frequent, but some biotypes have 35, 49, 56, ±63 and 84 chromosomes. In addition to this some aneuploid biotypes of *P. Crantzii* and *Tabernaemontani* have been observed and also an allopolyploid with $2n=62$, combining genomes of the two species.

(8) Pollen fertility in the material studied is strongly correlated with chromosome number, types with lower numbers being more sterile than »triploid» and »tetraploid» derivatives. This is in part due to reduplication of all genes necessary for the life of the pollen grain, in part to a change from a hybrid constitution to a more balanced allopolyploid constitution. Among natural biotypes of the three species studied pollen fertility is more or less reduced.

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THE MUTAGENIC ACTION OF ETHYLENE IMINE IN BARLEY

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INTRODUCTION

THE present investigation constitutes part of a series of studies of changes in the genetic material of higher plants, produced by chemicals. Among these, oxidants and alkylating agents (*e. g.*, mustards, methanesulfonic esters, and epoxides) have been investigated, as well as substances expected to act as antimetabolites in the bio-synthesis of nucleic acids (*cf.* EHRENBERG *et al.*, 1956 a, b).

In an earlier paper of this series (EHRENBERG and GUSTAFSSON, 1957) ethylene oxide was found, in comparison with ionizing radiations, to be a mutagenic agent of equal or even greater efficiency. — When different mutagenic agents are compared with regard to their *efficiency* as mutagenic agents, we must remember that this indicates the induced mutation rate relative to other biologic effects such as lethality and sterility, which cause an elimination of mutants. We thus mean the specificity at which the overall mutation rate is raised, and make no alludation to the mutagenic *effectiveness* of the agent, expressed as amount of mutations *per* amount radiation energy dissipated or *per* amount of chemical change induced in the material. There are, certainly, several very effective mutagenic agents which cannot be detected, since they are too inefficient, *i. e.*, they kill the material with a still greater effectiveness.

Although studies of mutagenic efficiency can give us valuable information as to the organization of the nuclear and cytoplasmatic material as well as data for estimations of eugenic hazards, the principal interest in finding very efficient mutagens is, consequently, a practical one: to give the plant-breeder as great a variation as possible at the lowest costs.

Although fast neutrons, per amount of energy dissipated in the tissue, produce mutations at a rate ca. 30 times higher than do X-rays, the

two agents are about equally efficient when the frequencies of viable mutations are compared at doses permitting corresponding percentages of surviving plants (EHRENBERG and NYBOM, 1954). Also ethylene oxide (EHRENBERG and GUSTAFSSON, 1957) and myleran [1.4-bis(methanesulfonyloxi)-butane; data unpublished] exhibited a somewhat greater or a similar mutagenic efficiency in barley, respectively. Since, however, these different agents have a specificity also with regard to the mutation types produced (*cf.* EHRENBERG *et al.*, 1956 a, b; NYBOM, 1956), they are complementary to each other in the practical use. — Especially in *Neurospora*, KØLMARK, GILES, and WESTERGAARD (see WESTERGAARD, 1957) have been able to demonstrate enormous differences between the sensitivities of individual genes to different mutagenic agents.

In the preceding paper of this series (EHRENBERG and GUSTAFSSON, 1957), ethylene oxide was found several times more efficient a mutagenic agent than the corresponding bi-functional substance, diepoxybutane, although the latter is about 300 times more effective. A double function has several times been claimed a necessity in order to give »radiomimetic» properties to an alkylating agent (*cf.* ALEXANDER and BACQ, 1955). The chief aim of the studies behind the definition of the word radiomimetic has been, however, the efforts to find nucleotoxic agents which effectively stop tumor growth. Also in barley, the diepoxybutane is more effective than the simple epoxide when considering growth inhibition. Obviously this occurs as a consequence of an interference with the nuclear material, which becomes disturbed in a way (*cf.* also ALEXANDER, 1952) that makes nuclear division impossible. Most gene mutations induced in the affected cells will therefore be eliminated, contrary to what happens after treatment with the simple alkylating analogue.

It seems at present to be generally agreed that alkylating »radiomimetic» agents exert their biological action chiefly by attacking the phosphate groups of the desoxyribonucleic acid (DNA) of the chromosomes. This was first shown by ELMORE *et al.* (1948) for mustard gas. ALEXANDER's (*l. c.*) hydrolysis experiments show that the alkyl group cannot be bound to the nitrogens of the purine and pyrimidine rings, and KØLMARK and GILES (1955) assume negative groups (*e. g.*, DNA phosphates) to be primarily attacked in the reversion of an adenineless *Neurospora* strain. They found the mutagenic effectiveness of substituted ethylene oxides (Fig. 1) to increase with increasing electronegativity of the substituent, R, at carbon 2, which, by electron displacement, leads

to an increasing positivity at carbon 3, where the ring opens at the reaction.

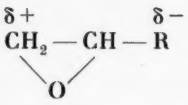

Fig. 1.

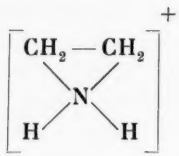

Fig. 2.

Bearing in mind the superiority, from the efficiency point of view, of the monofunctional alkylating compound and, in addition, the assumed role of the negatively charged phosphate groups as those being alkylated at mutation, a positive ion possessing the reactivity of ethylene oxide should have a greater mutagenic effectiveness than the latter. If further, which seems most probable, part of the lethal action of the compound is due to a more or less unspecific alkylation of, *e. g.*, amino and carbonyl groups in the cell, the positive ion would attack the negative phosphate groups more selectively than the neutral compound.

Since the nitrogen analogue of ethylene oxide, ethylene imine, is a strong base ($pK_s \simeq 10$), it is practically exclusively ionized in neutral milieu. (Formula of the ethylene iminium ion given in Fig. 2.) Being the simplest positive ion with alkylating capacity it was therefore tested on barley seeds under the conditions used for ethylene oxide. In accordance with the expectations, it was found to be, with respect to barley, the most efficient mutagenic agent hitherto known.

The ethylene imine was found already in 1948 by RAPOPORT to be »a remarkably powerful mutagenic agent», producing 10—15 % lethal mutations in *Drosophila*. RAPOPORT seems, however, not to have published his data. (*Cf.* his article of 1948, where they are mentioned shortly; see also SHKVARNIKOV, 1948, who found a treatment of *Crepis capillaris* seeds with ethylene imine to give different kinds of chromosome aberrations.) In *Neurospora* ethylene imine has nearly the same effect as ethylene oxide on the back-mutation rate of an adenineless strain (WESTERGAARD, 1957).

EXPERIMENTS

After a preliminary determination of toxicity (germination test of treated seeds), seeds of the two-rowed barley variety Bonus were treated for two hours in water solutions of ethylene imine, the pH of which had

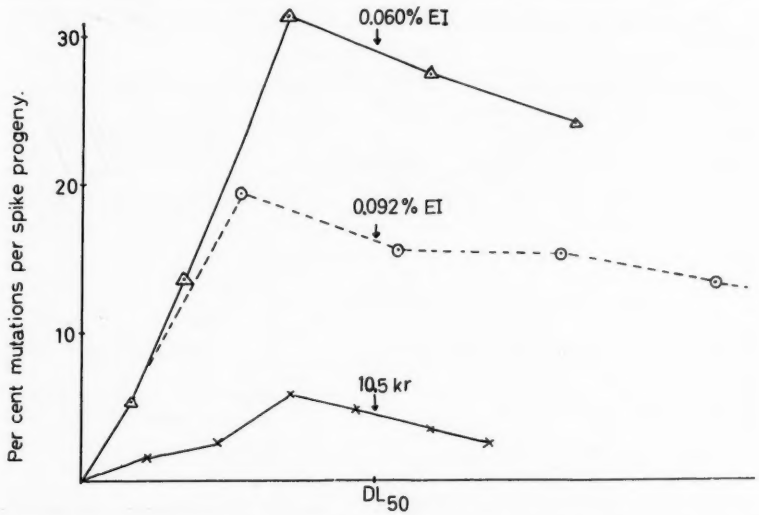

Fig. 3. Mutation rates induced by ethylene imine in dry seeds (triangles) and soaked seeds (circles, broken line) as a function of concentration giving 50 % lethality. For comparison: X-ray experiment from the year 1953.

been adjusted to 7.5 by addition of HCl. Resting seeds as well as seeds pre-soaked for 24 hours were treated. Since the latter were less sensitive in the toxicity test, they were treated at somewhat higher concentrations. (Due to the toxicity of fumes of ethylene imine — boiling point 57°C. — the handling must be done under conditions permitting good ventilation!) The treated seeds were sown in the field, and the mutation rate was estimated from the frequency of spikes segregating for chlorophyll deficient seedlings (*cf.* EHRENBERG and GUSTAFSSON, *l. c.*, and earlier publications).

In Fig. 3 the mutation rate found is given as a function of dose. In order to permit a comparison with radiation effects, the concentration (dose) giving 50 % lethality is set equal to unity on a linear scale. After treatment of resting seeds, around 30 % mutated spikes are obtained at certain concentrations (0.04—0.07 %). The compound is therefore 3—5 times more efficient a mutagenic agent than X-rays. The latter give in barley, in different years, an optimum mutation rate ranging between 5 and 10 % per spike progeny. In the Figure X-ray data from a typical experiment are given for comparison.

When a similar comparison is made between the sterilities induced by

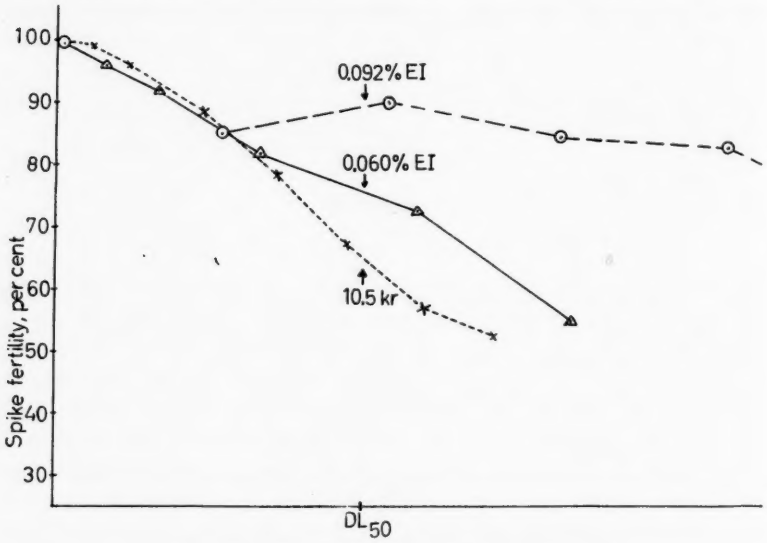

Fig. 4. Fertility data for the material in Fig. 3.

the imine and by X-rays (Fig. 4) the two agents do not differ. The mutagenic action of ethylene imine is therefore high also in relation to such chromosome rearrangements which lead to sterility.

In all respects the soaked seeds treated seem to be less sensitive. The DL_{50} was 0.092 % as compared to 0.060 % for the dry seeds. Mutation rate and sterility data are given in Figs. 3 and 4. When soaked seeds are treated by chemicals a low sensitivity is often simulated due to the fact that the water solution is imbibed more slowly than in a corresponding treatment of dry seeds. Since the solutions were not stirred during the treatment of the seeds, it is possible, especially under conditions of slow entrance of the agent, that a treated sample consists of seeds which have taken up variable amounts of the agent. A determination of the distribution of more and less affected seeds (*i. e.*, plants) will therefore give information as to whether the conditions for treatment were optimal or not. This question is especially important for the planning of the development of methods permitting still higher mutation rates to be reached. Since every seed gives rise to a plant with several spikes, about four of which were analyzed for mutations, the high mutation rates obtained permit an analysis of the distribution of plants with different numbers

TABLE 1. *Distribution of plants with different numbers of mutation cases.*

Material	x =	Number of plants with x mut. cases							Sum
		0	1	2	3	4	5	>5	
Soaked seeds, all concentrations	Found:	131	53	41	11	3	2	0	241
						5			
	Expected:	109.55	86.37	34.05	8.95	1.76	0.28	0.04	241
						2.08			
Average: 0.788 mut. cases/plant	$\chi^2 = 23.1084^{***}$; $p < 0.001$								
Resting seeds, 0.04—0.07 % ethylene imine	Found:	56	47	27	7	2	1	0	142
					10				
	Expected:	55.27	52.15	24.61	7.74	1.83	0.35	0.05	142
					9.97				
Average: 0.944 mutation cases/plant	$\chi^2 = 1.3014$; $0.8 > p > 0.7$								
Resting seeds, 0.02 % ethylene imine	Found:	100	33	5	2	3	0		143
					5				
	Expected:	93.34	39.82	8.49	1.21	0.13	0.01		143
					1.35				
Average: 0.427 mut. cases per plant	$\chi^2 = 12.9464^{(**)}$; $(0.01 > p > 0.001)$								

of mutation cases as a test for homogeneity of treatment. In Table 1 the actual distributions are compared with those expected from a Poisson distribution around the well-determined average (column 1). As a matter of fact the soaked seeds exhibit a significant over-representation of unaffected plants and of plants containing more than one mutation case. The lower effectiveness of the compound is therefore, certainly, due to a heterogeneous treatment, which can be made appreciably more effective. When the data for the two highest rates after treatment of dry seeds, giving in average 0.944 mutation cases per plant progeny, are analyzed in a similar way, a good agreement with the expected Poisson distribution is found. The same can be said of the lower rate obtained with 0.02 % ethylene imine: the high χ^2 is chiefly based on the low

numbers in the last class, and the significance of the deviation is therefore over-estimated by this method of computation.

It thus seems evident that, in the case of dry seeds, much cannot be gained by a homogenization of the treatment. In order to get still higher yields of mutations other ways have to be tried, *e. g.*, by utilizing the possibility that the reactions leading to lethality and to mutation may depend differently on temperature, or by making substitutions in the ethylene imine molecule.

Acknowledgment. — The present investigation was aided by a grant from the Agricultural Research Council.

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THE ORGANIZATION OF TELOMERES

IN SPECIES OF *SOLANUM, SALVIA, SCILLA, SECALE, AGAPANTHUS* AND *ORNITHOGALUM*

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I. INTRODUCTION

MOST segments of the chromosome are bipolar since after breakage they undergo reunion with other segments at both extremities. This is a primary condition for their survival in subsequent generations. Other segments are monopolar and only need to have this affinity satisfied at one extremity. These are the chromosome ends or telomeres (MULLER, 1940). Following breakage and reunion, a chromosome segment requires two telomeres and a kinetochore for subsequent survival which is significant in chromosome organization.

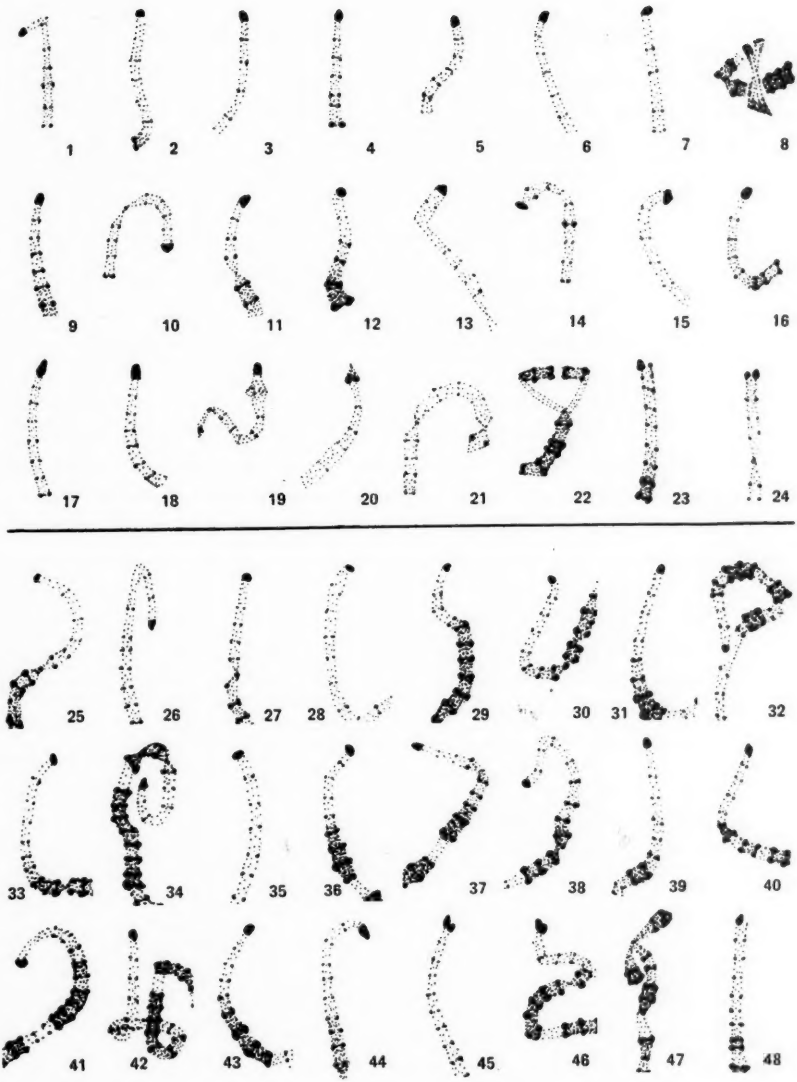
The kinetochore is now well known cytologically. Its pattern, organization and limits have been defined (LIMA-DE-FARIA, 1956, 1958). But what is the cytological differentiation corresponding to the monopolar segment? What are its limits, its pattern, and how is it organized? Since MULLER's work on *Drosophila*, a search has been made for the cytological counterpart of the genetic concept, but a pattern which could be considered of general occurrence has been missing.

In *Drosophila melanogaster* and *D. virilis* the ends show some differentiation (BRIDGES, 1935; WARTERS and GRIFFEN, 1950), but it is not a well defined pattern and is difficult to recognize in other species. In plants, however, a distinct pattern at the chromosome ends has been found in *Secale* (LIMA-DE-FARIA, 1949, 1952) and in tomato by BROWN (1949) and BARTON (1950). The present investigation was carried out to study the general occurrence of this pattern and to disclose its organization.

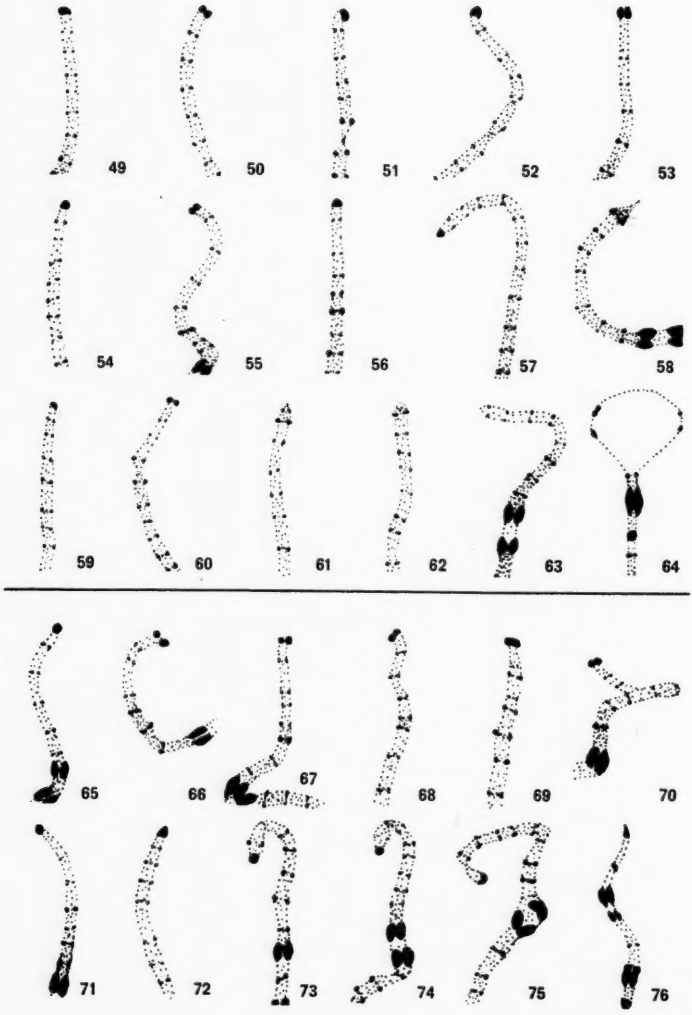
The materials and methods are summarized in Table 1. All figures are camera lucida drawings.

TABLE 1. *Materials and methods employed.*

Material	Haploid number	Stage studied	Source	Fixation	Staining	Mounting Medium	Family
Solanum lycopersicum (=Lycopersicon esculentum)	12	Pachytene	Variety Bonita	Acetic-alcohol 1 : 4	Aceto-carmine	Canada balsam	*Solanaceae*
Salvia viridis L. var. *horminum*	8	»	Botanical Garden, Lund	»	»	»	*Labiatae*
Scilla sibirica	6	Anaphase second pollen mitosis	Commercial variety	Acetic-alcohol 1 : 3	Aceto-orcein	temporary	*Liliaceae*
Agapanthus umbellatus	15	Pachytene	Botanical Garden, Lisbon	Acetic-alcohol 1 : 4	Aceto-carmine	Canada balsam	»
Ornithogalum virens	3	»	Seeds obtained from Dr. R. de V. Piennaar, South Africa	»	»	»	»
Secale cereale	7	»	Cross used by Lima-de-Faria (1952)	»	»	»	*Gramineae*



Figs. 1—48. *Solanum lycopersicum* ($2n=24$). Pachytene. The 24 telomeres of the chromosomes of one cell (Figs. 1—24) and of another cell (Figs. 25—48). ×3500.



Figs. 49—76. *Salvia viridis* ($2n=16$). Pachytene. The 16 telomeres of the chromosomes of a single cell (Figs. 49—64). Figs. 65—76, twelve telomeres from different cells. ×3500.

II. THE ORGANIZATION OF THE TELOMERES

A chromomere analysis of the above species was made, in most cases at pachytene, to permit an analysis of the pattern of the ends. Besides, the whole complement was studied in *Solanum lycopersicum*, *Salvia viridis*, *Ornithogalum virens* and *Secale cereale* in order to check the telomere of every arm.

The essential features of the pattern and organization of the telomeres in these species are the following:

A chromosome end may be divided into two distinct regions: (1) The terminal deep-staining structure called the *protelomere*, and (2) the weakly stained segment following it, the *eutelomere*.

In tomato, the chromosomes are terminated by a large deeply stained chromomere pair with a cone shaped appearance which is the protelomere. It has quite sharp limits (Figs. 1—48). Following this region is the chromosome segment where the chromomeres are the smallest and the fibrils are the thinnest and the most weakly stained. This segment or eutelomere is composed of one or two chromomeres and fibrils. It is at times so transparent that it becomes invisible. Such nuances are difficult to represent in the drawings (Figs. 151, 1—48). This region is more negatively heteropycnotic than the kinetochore, and is well delimited on the outer side but not always on the inner side. It shows, however, sharp limits in some chromosomes as will be seen in some of the other species described here. This compound pattern is present in 23 of the 24 ends and diverges only in the nucleolar organizing arm (Figs. 22 and 47). The protelomeres are different sizes in different arms. In some arms they are double the size of others (compare Figs. 1—3 with Figs. 17—19).

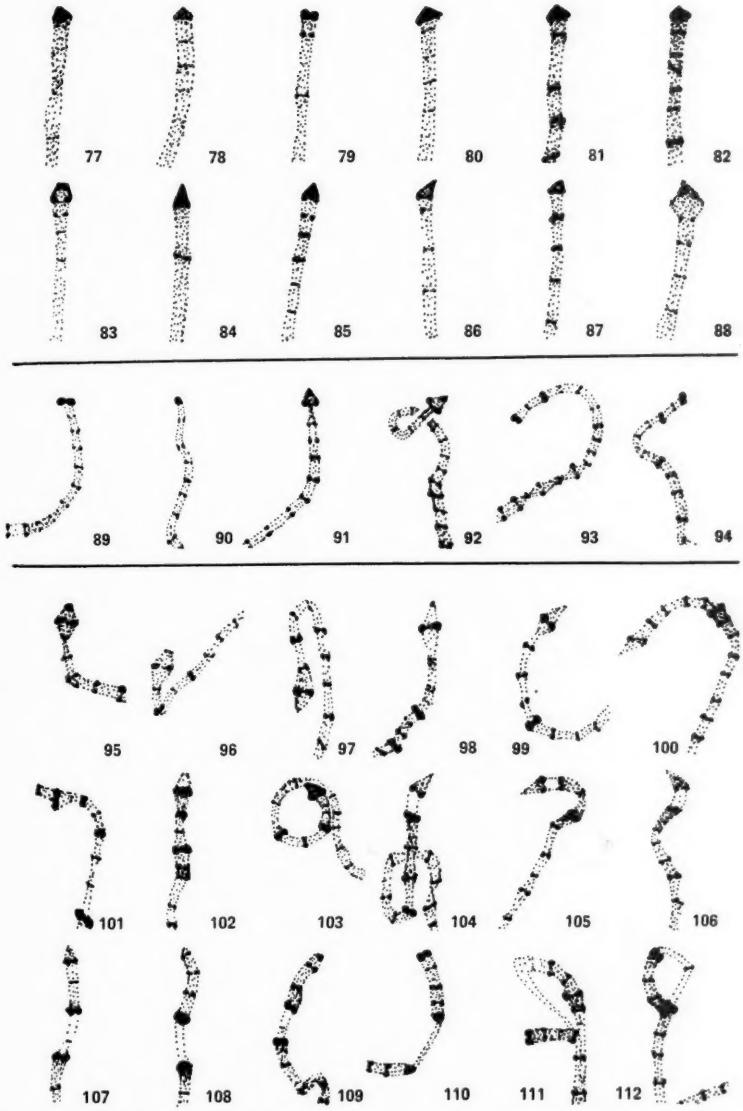
Salvia viridis which belongs to quite a different family (Labiatae) also has the same pattern. The ends of all the arms of a single cell are shown in Figs. 49—64. Distinct protelomeres are observed in 13 arms. In the remaining three only eutelomeres are apparently found. There is a regular pattern at all the ends. The nucleolar organizing arm also has a pro- and a eutelomere (Figs. 76 and 64). The protelomeres in *Salvia* also vary in size from arm to arm. The cone shape is particularly evident in Fig. 58. The protelomere is itself compound. Fig. 64 shows it to consist of three minor chromomeres (left chromosome of the nucleolar bivalent). When these chromomeres are close together they form the point shaped structure of Fig. 76 which shows the nucleolar organizing arm of another cell.

The chromosomes of *Scilla sibirica*, a Liliaceae, are partly despiralized at anaphase of the second pollen mitosis thus revealing their structure (Fig. 150). The protelomeres here have an accentuated triangular shape and are found at all 12 ends. On the other hand, differentiation into a eutelomere is indistinct at this stage (Figs. 77—88).

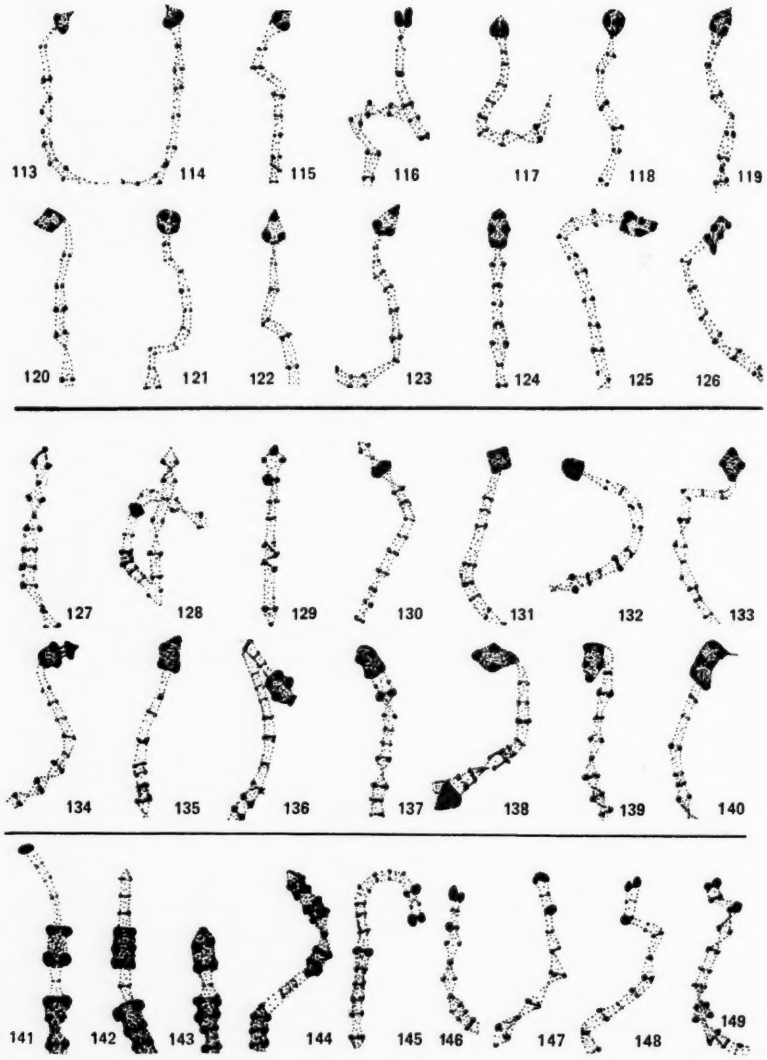
In *Agapanthus umbellatus*, another Liliaceae, the same pattern can be observed in many arms (Figs. 89—94). The protelomeres are distinctly compound (Figs. 91 and 92). The chromosomes of this species are particularly interesting regarding the structure of the eutelomeres. The very thin and weakly stained region that forms the eutelomere is particularly clear in Fig. 91. The eutelomeres can also be seen to be compound and are well delimited on both sides due to the presence of a large chromomere at one extremity and the protelomere at the other (Figs. 93 and 94).

A third Liliaceae, *Ornithogalum virens*, represents an intermediate case between sharply differentiated telomeres and undifferentiated ends as are found in many species and for this reason is included in this study. This species has only three chromosomes at pachytene and the six ends can be distinguished from each other. Two ends have an irregular chromomere pattern (Figs. 107—112). As in *Agapanthus*, the cone shape of the protelomeres results from a large chromomere pair situated on the inner side and a minute chromomere pair at the extremity (Fig. 98). The eutelomere is particularly well delimited in the left end of chromosome I (Figs. 104—106). It appears as a very weakly stained region having on the inner side a large chromomere (compare with Figs. 93 and 94 of *Agapanthus*).

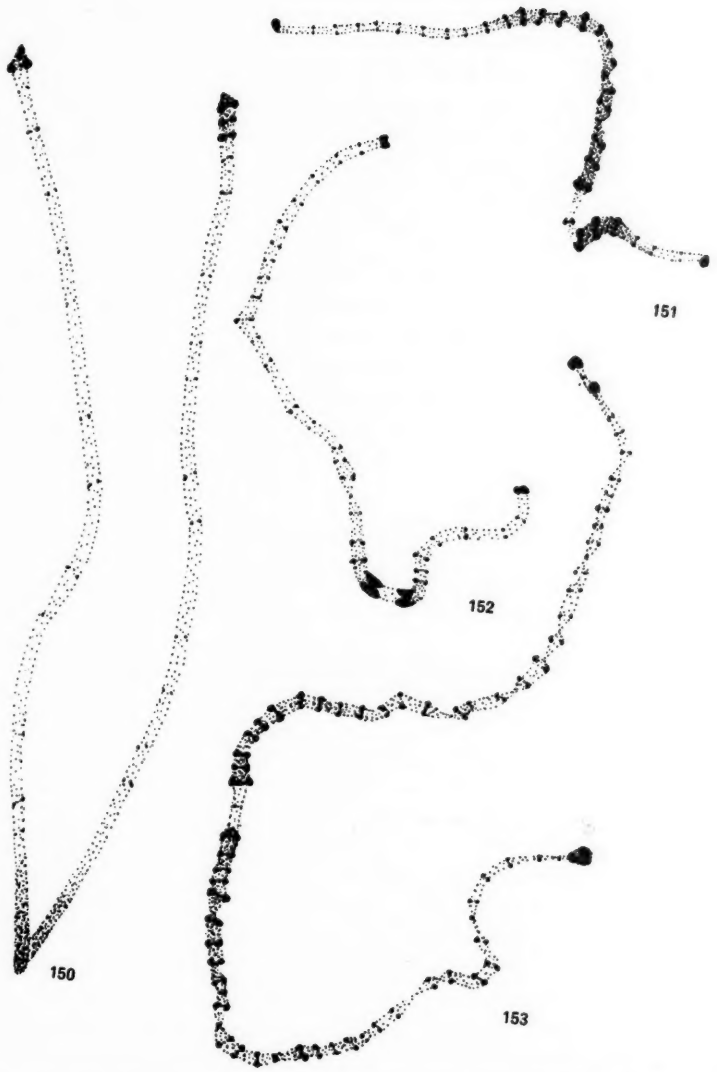
But, it is in *Secale cereale* that the organization of telomeres is best illustrated. The protelomeres are constituted by as much as three large chromomere pairs (Figs. 124—126, 136, 138—140) and are of different sizes in different arms as in *Solanum* and *Salvia* (Figs. 113—126). Of the fourteen chromosome ends of the normal complement of *S. cereale*, 11 show a typical differentiation into pro- and eutelomeres and 3 apparently terminate in a eutelomere (Figs. 127, 128, 130). The structure of the eutelomeres is particularly distinct in this species (Figs. 122, 123, and 153, the lower arm). These regions are thinner than any others of the complement. The best differentiation into pro- and eutelomeres is found at the left end of chromosome I (Fig. 137). The protelomere here is composed of two large chromomeres. Following it, the eutelomere is composed of a weakly stained fibril, in which a minute chromomere is sometimes found, and a large chromomere that delimits it quite well on



Figs. 77—88. *Scilla sibirica* (2*n*=12). Anaphase of the second pollen mitosis. Telomeres of different chromosomes. — Figs. 89—94. *Agapanthus umbellatus* (2*n*=30). Six telomeres of chromosomes from different cells at pachytene. — Figs. 95—112. *Ornithogalum virens* (2*n*=6). The six ends at pachytene: III R (Figs. 95—97), III L (Figs. 98—100), II L (Figs. 101—103), I L (Figs. 104—106), I R (Figs. 107—109), II R (Figs. 110—112). ×3500.



Figs. 113—149. *Secale cereale* ($2n=14$). Pachytene. Figs. 113—126. Telomeres from different arms showing variation of size. — Figs. 127—140. The 14 telomeres of the complement from one cell. — Figs. 141—143. Telomeres of the right arm of standard B chromosome in a Turkish strain, and in the varieties Wasa and Östgöta Gråråg, respectively. — Fig. 144. Left arm of the same B chromosome (Wasa). — Figs. 145—149. Telomere of right arm of chromosome IV in five different cells. ×3500.



Figs. 150—153. Chromosomes of *Scilla sibirica* (Fig. 150), *Solanum lycopersicum* (Fig. 151), *Salvia viridis* (Fig. 152), and *Secale cereale* (Fig. 153). Fig. 150, second anaphase of the pollen mitosis, Figs. 151—153, pachytene. ×3500.

the inner side. Thus, in *S. cereale*, both pro- and eutelomeres are compound structures. The right end of chromosome III apparently lacks a protelomere. It is differentiated as it possesses only a eutelomere delimited on the inner side by a large chromomere (Fig. 130). This is undoubtedly the case in the standard B chromosome of the Wasa variety where the right arm ends in a sequence of three very small chromomeres (Fig. 142). Confirmation that this terminal weakly stained region is a eutelomere is obtained in other strains of rye where a typical cone shaped protelomere terminates the arm (Fig. 141). But there are other strains of rye, like Östgöta Gråråg, where the right arm ends in a sequence of large chromomeres which is homologous with the protelomeres of other arms (Fig. 143). Following this protelomere, there is a weakly stained region with minute chromomeres which is homologous with the eutelomeres of the other chromosomes. The standard B has two types of telomeres: a primary and a secondary one which both perform a monopolar function.

III. THE DELETION OF TELOMERE SEGMENTS

For the knowledge of the organization of the telomere it is particularly valuable to know which segments may be deleted without loss of its essential property.

Pachytene analysis in *Solanum lycopersicum* revealed the occurrence of terminal deficiencies, and these cases were carefully studied. These were considered reliable only where the ends of a chromosome pair showed a large and a small chromomere, and where the two chromosomes were seen running together side by side. Deficiencies involving part or even the whole protelomere were found in different arms and in several cells (Figs. 23, 24, and 48).

In tomato it has been difficult to ascribe the deficiencies in different cells to the same chromosome end due to the difficulty of recognizing some of the equal armed chromosomes. But in *Secale cereale* where the ends can be easily recognized, this has been possible. The right end of chromosome IV shows a deficiency of part of the protelomere and a deletion at the eutelomere in all studied cells of the same plant. The chromomere pair that constitutes the protelomere is composed of one large and one small chromomere. The same is true of the large chromomere pair that delimits the inner side of the eutelomere (Figs. 145—149). Thus, both pro- and eutelomeres may lose segments without the survival of the chromosomes being affected.

IV. VARIATION OF THE TELOMERE PATTERN IN RELATION TO GENETIC DIFFERENTIATION

From the preceding description it is apparent that instead of having typical telomeres some arms or chromosomes showed an irregular chromomere pattern. Such a pattern is not distributed at random throughout the complement, but occurs only in particular chromosomes or arms. This difference can only be established when the chromosome pattern follows definite rules, and when all the chromosomes can be analysed separately as is the case in *Solanum*, *Salvia*, and *Secale*.

Of the 24 ends of *S. lycopersicum* chromosomes, only one has a deviating pattern. It shows a seriation of large chromomeres and deeply stained fibrils followed by a weakly stained region. This end is of the nucleolar organizing arm (Figs. 22 and 47).

Similarly, in *Salvia* it is also only the nucleolar organizing arm which shows a deviating pattern although there is a typical telomere at the end (Figs. 64 and 76).

In the standard B chromosome of *Secale* the pattern is irregular in the left arm and constituted by large chromomeres. There is no differentiation which resembles a pro- or eutelomere (Fig. 144). Throughout the long arm, the pattern is also irregular as compared with the 14 arms of the normal complement. As described above, this arm has two types of telomeres. The primary one is found in most strains (Fig. 141). In the variety Östgöta Gråråg, only the secondary telomere may be present (Fig. 143).

Consequently, in *Solanum*, *Salvia*, and *Secale*, the arms and the ends which show a deviating pattern are only those which are either associated with the nucleolus or are part of B chromosomes. The first type of chromosome is involved in the synthesis of RNA while the second is known for its genetic inertness or sub-inertness. The modification of the chromomere pattern of telomeres is thus correlated with a genetic specialization.

V. DISCUSSION

When MULLER formulated the telomere concept it had only a genetic basis and its transposition to cytological terms remained unclear for many years. Similar to the kinetochore which has been considered throughout the thirties and fourties as a »gap» or a »constriction», the telomere has had a still more vague denomination; it was just an »end».

MULLER conceived the telomere as a segment containing the »telo-

gene» or genes responsible for the monopolar character. These genes were situated at the chromosome tip and he thought the telomere would thus consist of only one or two bands.

The work done during the same period on *Drosophila* and maize (McCLINTOCK, 1931), confirmed MULLER's interpretation of the behaviour of chromosome ends as monopolar structures. But at the same time, it caused doubt about the concept of the telomere as confined only to the very end. Many cases of terminal deficiencies were reported where the chromosomes survived after the loss of as many as 10 to 11 bands (DEMEREC and HOOVER, 1936). These and other results led CATCHESIDE and LEA (1945) to deny the existence of telomeres and to consider MULLER's concept as incorrect.

MULLER tried to answer this criticism and the contrary evidence by denying the terminal nature of the deficiencies (MULLER, 1940; MULLER and HERSKOWITZ, 1954). According to him, these may represent only cases where the distal break occurred so near to the end as not to be demonstrable. Any of these deficient chromosomes that survived would still contain a telomere that would be so small as to be undetectable by ordinary cytological methods. His interpretation is undoubtedly an attempt to have the results fit his original concept of the telomere as a single gene or a few genes located in one band. Such a view is untenable in light of the extent and accuracy of the results on terminal deficiencies (STADLER, 1939; MULLER and PROKOFYEVA, 1935; DEMEREC and HOOVER, 1936; KIKKAWA, 1938; SUTTON, 1943).

The above cytological data support a concept of the telomere which harmonizes the monopolar behavior of ends with the work on terminal deficiencies. The telomere is a compound structure consisting of several differentiated segments and not a single band or chromomere at the tip of the arm. Thus, it can lose segments without affecting its main function. In rye it is composed of at least 8 segments: 4 chromomeres and 4 fibrils. All of them seem to possess the essential property of monopolarity as revealed by viable terminal deficiencies found in chromosome IV and in the standard B chromosome. Similar viable deficiencies involving different segments of the telomere of the short arm of chromosome 9 of maize were reported by McCLINTOCK (1944). In this respect, the telomere is like the kinetochore which is also a highly differentiated compound structure. Half to one third of its elements contain the essential property of active mobility and are able to perform it alone (LIMA-DE-FARIA, 1955).

On the other hand, telomeres are not the only monopolar structures.

Kinetochores of animal chromosomes also possess it. In plants, kinetochores usually need to combine with two arms to form a stable chromosome. The kinetochore in animal species may be strictly terminal in all the chromosomes of the complement, *e. g.* the crustacean *Ulophysema öresundense* (MELANDER, 1950), the mouse (TJIO and LEVAN, 1954), and the grasshopper *Mecostethus grossus* (LIMA-DE-FARIA, 1956). In these chromosomes the kinetochore is a monopolar element, and its appearance is strikingly similar to that of a eutelomere.

The cases reported by MCCLINTOCK (1941, 1942) of permanent healing of median segments of maize chromosomes in sporophytic tissues do not have to be taken as contradiction of the telomere concept. The healing took place in many regions but mostly at segments which originally represented telomeres. Her results may be interpreted that other regions of the chromosome manifest monopolarity under certain physiological conditions. This is also the case of the telomeres of both rye and maize when they manifest active mobility like kinetochores on the spindle. This property of telomeres is also only manifested in one tissue, *viz*: the pollen mother cells (PRAKKEN and MÜNTZING, 1942; RHOADES and VILKOMERSON, 1942).

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SUMMARY

(1) A chromosome end may be divided into two distinct regions: the terminal deep-staining structure called the *protelomere*, and the weakly stained segment following it, the *eutelomere*.

(2) The protelomere has sharp limits. The eutelomere is well delimited on the outer side but not always on the inner side.

(3) The protelomere is usually constituted by one to three large chromomeres and deep staining fibrils, and the eutelomere by one to two minute chromomeres and very transparent and thin fibrils. The whole telomere may consist of as many as eight segments.

(4) This compound pattern was found in six species belonging to the *Solanaceae*, *Labiatae*, *Liliaceae*, and *Gramineae*.

(5) Terminal deficiencies involving part or the whole protelomere were found at pachytene in *Solanum lycopersicum*. The right end of

chromosome IV of *Secale cereale* showed a deficiency of part of the protelomere and a deletion at the eutelomere.

(6) The standard B chromosome of rye shows two types of telomeres: a primary and a secondary one. In the nucleolar arm of *Solanum lycopersicum* and *Salvia viridis* the pattern is irregular while in tomato this is the only arm which does not show a telomere.

(7) MULLER's telomere concept may be considered as well established regarding the behavior of chromosome ends as monopolar structures. But his concept of the telomere as consisting of one single terminal band or chromomere is inconsistent with the results on deficiencies. These results, however, become intelligible when cytological analysis reveals the telomere to be a compound structure.

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INHERITANCE OF LOBED LEAF FORM IN *LACTUCA*

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I. INTRODUCTION

LEAVES of *Lactuca* species show great diversity of form, varying from lobed leaves with deep incisions, to leaves which are almost entire, *i. e.*, which practically lack any kind of indentation of the margin. The aspect of leaf form to be considered in this paper is that of lobed versus non-lobed. No attention has been paid to differences in serration of the margin.

Lobed and non-lobed forms are known in the garden lettuce as well as in the wild species of the *Serriola* section of *Lactuca*, to which *L. sativa* L. belongs. Among the wild species only two, *L. serriola* L. and *L. altaica* FISCH. and MEY. — the latter possibly only a subspecies of *L. serriola* — give fertile hybrids with *L. sativa*. They are therefore the only two, which are of interest in an investigation like this, which is concerned primarily with the genetics of cultivated lettuce.

Non-lobed leaves are prevalent in *L. sativa*, being found in most varieties of cabbage, cos and asparagus lettuce. Lobed leaved varieties are found mainly in the leaf lettuce group. They are often referred to as »oakleaved» lettuces because of a certain similarity between the young leaves and those of the oak. When fully developed the leaves exhibit a peculiar unsymmetrical type of lobing with deep incisions. This type of lobing is illustrated in Fig. 4.

The wild species *L. serriola* L. occurs in two forms: *f. typica* ROUY, with lobed leaves and *f. integrifolia*, BOGENHARD, with non-lobed leaves. The type of lobing in *f. typica* is markedly different from that described for the »oakleaved» lettuce. There is considerable variation in the depth of the incisions, but the leaves are usually highly symmetrical. The non-lobed form is illustrated in Fig. 1 and the lobed form in Fig. 3. A rare form, *L. serriola, f. plicata*, reported from Greece and Italy, has a pattern of lobing very similar to that of the »oakleaved» varieties of *L. sativa*.

The taxonomical position of *L. altaica*, FISCH. and MEY., is uncertain

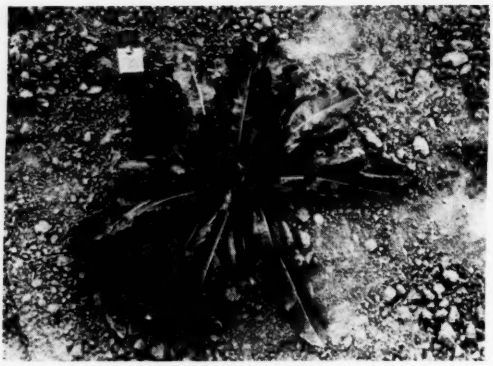

Fig. 1. Sc. H (non-lobed *L. serriola*)

and very little appears to be known about the variation within this species. The form used in this study was obtained from the Royal Botanic Gardens, Kew, England, and it had lobed leaves of a type similar to that of *L. serriola f. typica.* A specimen of this form is illustrated in Fig. 2. THOMPSON, WHITAKER and KOSAR (1940) worked with a form of *L. altaica* originating from Egypt. Judging by their illustrations, this form had non-lobed leaves.

The inheritance of leaf form in *Lactuca* has previously been studied by DURST (1930), ERNST-SCHWARZENBACH (1936) and WHITAKER (1950). Their F_2-results are summarised in Table 1. DURST crossed a lobed form of *L. serriola* with a non-lobed form of the same species and also with the two cabbage lettuce varieties May King and Big Boston and found that the lobed condition was dominant in F_1. The cross with the non-lobed *serriola* gave in F_2 a segregation which did not differ significantly from 3 : 1, but the crosses with the two cabbage lettuce varieties gave a much higher proportion of non-lobed plants in F_2. The result of the cross with May King was in very good agreement with a 9 : 7 ratio, but in the cross with Big Boston there was significant heterogeneity between the two families, one agreeing with a 9 : 7 ratio, the other reasonably well with 3 : 1.

On the strength of these F_2-results and his F_3-data, DURST concluded that two dominant complementary genes, U_1 and U_2, are necessary to produce the lobed condition. In order to account for the 3 : 1 segregation in the cross between lobed and non-lobed *L. serriola*, he assumed that the latter form has one of the dominant genes. The two cabbage lettuce varieties were supposed to be homozygous for both the recessive alleles.

TABLE 1. *F_2-results obtained by other authors.*

Author	Non-lobed parents	Lobed parents						
		Oakleaved *sativa*			Lobed *serriola*			
		lobed	non-lobed	χ^2 3 : 1	lobed	non-lobed	χ^2 3 : 1	χ^2 9 : 7
DURST	May King				402	317		0.034
»	Big Boston				[1] 141	93	27.128***	1.526
					237	98	3.233	28.606***
»	Non lobed serriola				73	31	1.282	
SCHWARZENBACH	Hochheimer Dauerkopf				49	39		0.012
»	Blutroter Kochsalat							
»	Transport							
»	Stuttgarter	55	15	0.476				
»	Wunder der 4 Jahr.							
»	Blutroter Kochsalat	41	6	3.752				
»	Non lobed serriola	110	34	0.148	381	120	0.293	
»	Stuttgarter							
»	Danhäuser				438	202	14.700***	38.630***
»	Transport							
WHITAKER	Paris White Cos	109	30	0.866				
»	Imperial	88	31	0.070				

*** significant at the 0.001 level

[1] χ^2 for heterogeneity between families 11.284: $P < 0.001$

Fig. 2. *L. altaica*

Fig. 3. Sc. S. Afr. (lobed *L. serriola*)

u_1 and u_2. DURST himself points out that he has not performed the crucial test for the existence of two complementary genes for lobing, namely to produce a lobed F_1 by crossing two non-lobed segregates.

ERNST-SCHWARZENBACH used as lobed leaved parents in her crosses partly a lobed form of *serriola* and partly an »oakleaved» form of *sativa*, namely the leaf lettuce variety »gelber krauser Schnittsalat». As non-lobed parents she used a non-lobed form of *serriola* and some varieties of cos and cabbage lettuce. She has pooled results from different crosses in the way shown in Table 1 because the number of plants in some of the crosses was very small. The results of ERNST-SCHWARZENBACH's crosses involving lobed *serriola* agree well with DURST's results. The proportions of the two leaf form types in the cross lobed × non-lobed *serriola* is a good fit for 3 : 1, but in the crosses where the non-lobed parent is a *sativa* variety the proportion of recessives is much higher than $^1/_4$. The total of the three crosses with Stuttgarter, Danhäuser and Transport is intermediate between 3 : 1 and 9 : 7, but it is not possible to judge the significance of this without the knowledge of the ratios in the individual crosses. The results of the crosses with »gelber krauser Schnittsalat» as lobed parent do not show any serious deviations from the monohybrid ratio. Crosses between »gelber krauser Schnittsalat» and lobed *serriola* produced only lobed plants in F_1 and F_2.

Fig. 4. Eichenblättriger.

ERNST-SCHWARZENBACH concludes that the mode of inheritance of lobed leaves in *L. serriola* and *L. sativa* has not yet been fully explained and she suggests that the discrepancies may be due to external influences.

WHITAKER used as lobed parent the *sativa* variety Oak Leaf. This is a leaf lettuce like ERNST-SCHWARZENBACH's »gelber krauser Schnittsalat» and from the illustrations of the two authors it is obvious that the type of lobing is the same in the two varieties. WHITAKER's crosses with Paris White Cos and Imperial gave results in good agreement with a 3 : 1 ratio. WHITAKER discusses DURST's hypothesis of inheritance of lobing on the basis of two complementary genes, and he suggests that in DURST's material there may have been only one basic gene for lobing, but that the monohybrid ratios were disturbed by the segregation of genes for serration of the leaf margin.

This paper presents the results of a number of crosses made with a view to throwing further light on the problem of inheritance of lobing in lettuce.

II. MATERIAL AND METHODS

Ten different forms with lobed leaves and sixteen with non-lobed leaves have been used as parents. Information about name, leaf form and origin of the parents is found in Table 2. The two unidentified

TABLE 2. *Parental lines.*

Abreviation	Full name	Leaf form	Origin
Sc. L	*L. serriola* L. *f. typica* ROUY	lobed	Wild population, Lund, Sweden
Sc. M	» » » »	»	» » , Malmö, Sweden
Sc. R	» » » »	»	» » , Romford, England
Sc. S. Afr.	» » » »	»	» » , South Africa
Sc. I	» » » »	»	Botanic Garden, Lund, Sweden
Fl. 23	—	»	Segregate from R. Liebl.×Sc. I
Altaica	*L. altaica*, FISCH and MEY	»	Royal Botanic Gardens, Kew, England
Oakl.	Oakleaved	oakleaved	Commercial leaf lettuce variety
Eichenbl.	Eichenblättriger	»	» » » »
Oakl. tete	—	»	Segregate from Tezier×Oakl.
R. rouge	Romaine rouge d'hiver de Vendée	non-lobed	Commercial cos variety
Paris	Paris White Cos	»	» » »
K. driv	Kejsar driv (syn. Emperor forcing)	»	» forcing variety
Forcing	(Name unknown)	»	» » »
Merv.	Merveille des 4 saisons	»	» cabbage variety
Asiat.	Asiatisk (syn. Deacon)	»	» » »
Berl.	Berliner	»	» » »
Maik.	Maikönig	»	» » »
R. Liebl.	Rudolph's Liebling	»	» » »
Laur.	Laurenzianer	»	» » »
TT tete	—	»	Segregate from Rom. royale×Tezier
t	—	»	Segregate from K. driv×Laur.
Asp.	Asparagus lettuce	»	Commercial variety
Sc. H	*L. serriola L. f. integrifolia*	»	Wild population, Harwich, England
SP	(Identity unknown)	»	Botanic Garden, Palermo, Italy
SL	(» »)	»	» » , Lund, Sweden

forms, SP and SL, have been referred to in an earlier paper (LINDQVIST, 1956). It is now known that they are not identical with ALLIONI's *L. augustana*, which is a non-lobed form of *L. serriola* with reflexed involucre, whereas SP and SL have the erect involucre. Both have non-lobed leaves. Very closely related forms with lobed leaves have recently been obtained by the author, but no results from crosses with these forms are as yet available. In order to distinguish between the »oak leaf» type of lobing and the type found in *serriola* and *altaica*, the name

»oakleaved» will be retained for the former and the name »lobed» will be used only for the latter. An account of the technique of crossing and of growing the plants has been given in an earlier paper (LINDQVIST, 1956).

The lettuce is susceptible to a number of seedling diseases and it has not always been possible to avoid losses especially in early sowings. A certain number of plants usually also died after having been transplanted to the field. Because of these hazards it was necessary to sow a number of seeds well in excess of the required number of plants, usually twice as many. When germination was good and seedling losses small the number of available seedlings was often too high for all of them to be planted out. This means that usually less than half of the seeds have given classified plants.

III. EXPERIMENTAL

1. F_1-generations

As was to be expected from the work of other authors both oakleaved and lobed were dominant to non-lobed. It was also found that oakleaved was dominant to lobed, a fact which has not been reported before. The completeness of the dominance was difficult to judge because genes affecting the general shape and texture of the leaf were involved in most crosses. In all cases the dominance was, however, too strong to allow identification of homozygotes and heterozygotes in segregating families. Figs. 5—8 show F_1-hybrids of crosses between various oakleaved, lobed and non-lobed forms.

2. F_2-generations

F_2-results of the various crosses are presented in Tables 3—7. In cases of significant deviations from 3 : 1, heterogeneity χ^2's have been calculated according to the BRANDT and SNEDECOR formula. (MATHER, 1938, Chapter II.) The levels of significance of χ^2's have been indicated with asterisks (* = 0.05—0.01, ** = 0.01—0.001, *** = < 0.001).

Where the χ^2 for heterogeneity between families is significant, results of the individual families are given instead of totals for the crosses. Two χ^2's for heterogeneity between crosses have been calculated, one including all crosses, the other only those with good agreement between families.

One very significant feature about the F_2-data is the fact that only the two parental leaf form characters appear in F_2 whether the cross

Fig. 5. F_1 R. rouge×Sc. S. Afr.

is of the type oakleaved×lobed, oakleaved×non-lobed or lobed×non-lobed. This indicates that the genes for the three characters are multiple alleles. Therefore one would expect 3 : 1 ratios in all the crosses, but such is obviously not the case. There are very large deviations from the monohybrid ratio and in some crosses there is an excess in the dominant class, in others in the recessive class.

Crosses between oakleaved and lobed. — In the crosses oakleaved×lobed and lobed×oakleaved (Table 3) there is everywhere an excess of oakleaved plants. The χ^2 for heterogeneity between crosses is significant, the excess in the Oakleaved×Altaica cross being less pronounced than in the remaining four. That these four crosses are in good agreement is shown by the following χ^2 analysis which also proves that it is without importance for the segregation ratios whether the male or the female parent has the oakleaved character.

Heterogeneity between	χ^2	DF	P
crosses of reciprocal types	1.235	1	0.5—0.7
» » same type	2.337	2	0.2—0.3
Total:	3.572	3	0.3—0.5

The excess of oakleaved is very large and in some of the crosses the ratios do not differ significantly from 15 : 1.

Crosses between oakleaved and non-lobed. — Varying ratios, most of

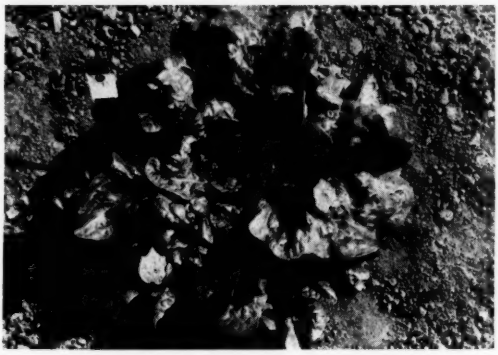

Fig. 6. F_1 Laur.×Altaica

them not agreeing with any simple mendelian ratio, are found in the crosses oakleaved×non-lobed and non-lobed×oakleaved (Table 4). The χ^2 test shows significant heterogeneity between crosses. In some there is

TABLE 3. *F_2-results of crosses between oakleaved and lobed.*

Oakleaved × lobed						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3 : 1
			oakleaved	lobed		
Oakl.×Altaica	3	0.294	238	57	295	5.072*
Oakl.×Sc. S. Afr.	2	0.448	156	13	169	27.000***
Oakl.×Fl. 23	4	1.174	353	39	392	47.361***
Total			747	109	856	
χ^2 for deviation from 3 : 1:			68.692***			
χ^2 for heterogeneity between crosses:			18.124***			

Lobed × oakleaved						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3 : 1
			oakleaved	lobed		
Sc. M×Oakl.	3	2.532	264	34	298	29.356***
Sc. S. Afr.×Oakl.	2	0.145	124	10	134	21.980***
Total			388	44	432	
χ^2 for deviation from 3 : 1:			50.568***			
χ^2 for heterogeneity between crosses:			1.574			

Fig. 7. F_1 Oakl.×Sc. S. Afr.

a highly significant excess in the oakleaved class compared with a 3 : 1 ratio, in others a significant deficiency. Among the former are crosses with the two cos lettuce varieties Romaine rouge and Paris White Cos. The cabbage lettuce varieties do not behave uniformly, some giving normal 3 : 1 ratios others deviating significantly in one direction or the other. In some cases there are significant differences also between families of the same cross.

The heterogeneity of the data restricts the comparison between crosses oakleaved×non-lobed and non-lobed×oakleaved. The following χ^2 analysis, however, indicates that the direction of the cross has little or no influence on the F_2 ratios.

Heterogeneity between	χ^2	DF	P
Forcing×Oakl. and reciprocal	2.664	1	0.1—0.2
K. driv×Oakl. and Eichenbl.×K. driv	0.802	1	0.3—0.5

Crosses between lobed and non-lobed. — The individual crosses of the types lobed×non-lobed and non-lobed×lobed also behave very differently, a fact which is reflected in large χ^2's for heterogeneity between crosses. Often there is significant heterogeneity also between families of the same cross.

In some crosses the ratios are in good agreement with 3 : 1, in others there is a more or less pronounced excess of the recessive type. The

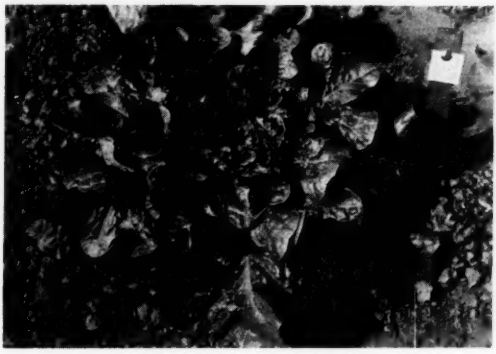

Fig. 8. F_1 Paris×Oakl.

extreme case is the small F_2 of the cross TT te te×Fl. 23 where the recessive type actually out-numbers the dominant. It is a remarkable fact that among the 33 crosses between lobed and non-lobed forms there is not one which shows a significant excess of the dominant lobed type. As in the case of the crosses between oakleaved and non-lobed, heterogeneity makes it difficult to compare the results of crosses made in different directions. Only the reciprocal crosses between Sc. S. Afr. and Maik. are suitable for such a comparison. They show satisfactory agreement, the χ^2 for heterogeneity being 2.541 (P=0.1—0.2).

The results in Table 5 suggest that the anomalities of the F_2-ratios are determined genetically and influenced both by lobed and non-lobed parents. These facts emerge more clearly from the following tabulation where the crosses have been divided into groups according to lobed and non-lobed parentage and classified according to whether they give normal 3 :1 segregation or an excess in the recessive class, significant at least at the 5 % level.

Non-lobed parent	Lobed parent	No. of crosses: Normal segregation	No. of crosses: Excess of non-lobed
Sc. H	×*altaica*	1	0
» »	×*serriola*	3	0
Cos lettuce	×*altaica*	2	0
» »	×*serriola*	3	1
Cabbage lettuce	×*altaica*	2	4
» »	×*serriola*	0	10
SL	×*altaica*	0	1
»	×*serriola*	1	0
SP	× »	0	1

TABLE 4. *F_2-results of crosses between oakleaved and non-lobed.*

Oakleaved × non-lobed						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3:1
			oakleaved	non-lobed		
Oakl.×R. Rouge	3	13.925***	132	10	142	24.423***
			58	17	75	0.218
			66	19	85	0.360
Oakl.×Forcing	6	6.995	404	80	484	18.523***
Oakl.×Laur.	1	—	208	50	258	4.346*
Oakl.×Merv.	3	6.760**	69	27	96	0.500
			57	37	94	10.340**
			76	22	98	0.340
Oakl.×K. driv	1	—	293	148	441	17.234***
Oakl.×»t»	1	—	94	37	131	0.735
Oakl.×Sc. H	5	6.449	294	76	370	3.924*
Oakl.×SP	3	2.329	314	112	426	0.379

χ^2 for heterogeneity between crosses: 1. All crosses: 63.565***

2. Homogeneous crosses: 45.052***

Non-lobed×oakleaved						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3:1
			oakleaved	non-lobed		
R. rouge×Oakl.	5	13.278***	72	5	77	14.065***
			110	14	124	12.430***
			121	19	140	9.752**
			109	31	140	0.610
			93	23	116	1.655
Forcing×Oakl.	3	0.686	138	39	177	0.831
Paris×Oakl.	3	5.704	357	65	422	20.730***
Asp.×Oakl.	2	3.347	143	44	187	0.216
TT te te×Oakl.	3	1.549	289	68	357	6.746**
Berl.×Oakl.	7	1.928	501	162	663	0.113
Asiat.×Oakl.	3	0.812	251	113	364	7.092**
K. driv.×Eichenbl.	1	—	278	123	401	6.884**
R. rouge×Oakl. te te	1	—	65	20	85	0.098

χ^2 for heterogeneity between crosses: 1. All crosses: 64.297***

2. Homogeneous crosses: 42.142***

For the purpose of this analysis Asp. has been included in the cabbage lettuce group as it behaves in the same way, and the segregate lobed line Fl. 23 has been included with the *serriola* forms. Among the lobed parents the *serriola* forms have given a much higher proportion of abnormal F_2-ratios than *altaica* and among the non-lobed parents it is particularly the cabbage lettuce varieties which have produced F_2-ratios deviating from 3 : 1. In fact all the 10 crosses between cabbage lettuce varieties and lobed *serriola* forms have given a significant excess of the non-lobed type.

There is a marked difference in the behaviour of the cabbage and the cos varities but no general conclusions must be drawn from this fact as the cos lettuces are represented by only two varities. It is remarkable that the non-lobed *serriola* form has given normal 3 : 1 segregations in all crosses, a fact which agrees with the results of DURST and ERNST-SCHWARZENBACH.

Crosses between lobed forms. — The crosses between different lobed forms produced only lobed plants in F_2 (Table 6). Although there is a distinct difference in leaf shape between Altaica and the other lobed forms it was not possible to distinguish different types of lobing in F_2. The difference in leaf shape between the parents is due to genes influencing other aspects of the appearance of the leaf such as shape of apex, length, width and serration and not to different alleles for lobing.

No F_2-results are available for crosses between the different lobed *serriola* forms (including Fl. 23) but the parallel behaviour of these forms in crosses with Oakl., Altaica and various non-lobed forms, leaves little doubt that they carry the same genes for lobing.

Crosses between non-lobed forms. — It has not been possible to make all the 120 crosses which are possible between the 16 non-lobed forms used in this investigation. F_2-data are available from 17 crosses — see Table 7 — and these have given only non-lobed plants. In addition F_1-hybrids which were all non-lobed have been obtained from the following six crosses: Berliner × Paris, Paris × Asp., Paris × Sc. H, R. rouge × Sc. H, Merv. × Sc. H and SL × SP.

Although there is no evidence in these crosses for the existence of complementary genes for lobing, the possibility cannot be completely excluded because of the incompleteness of the data.

In some of these crosses evidence was found of modifying genes which can produce a slight tendency to lobing. This was the case particularly in the cross Berl. × SL where a few plants were found which

TABLE 5. *F_2-results of crosses between lobed and non-lobed.*

Lobed × non-lobed						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3 : 1
			lobed	non-lobed		
Altaic × Asp.	2	5.066*	118	39	157	0.002
			91	53	144	10.704**
Altaica × Sc. H	5	3.469	344	110	454	0.144
Fl. 23 × R. rouge	3	0.376	205	86	291	3.218
Sc. S. Afr. × Maik.	2	0.387	60	46	106	19.132***
Sc. S. Afr. × Sc. H	3	0.363	378	136	514	0.584
Sc. L × SP	3	4.819	173	97	270	17.190***

χ^2 for heterogeneity between crosses: 1. All crosses: 23.775***
2. Homogeneous crosses: 23.606***

Non-lobed × lobed						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3 : 1
			lobed	non-lobed		
R. rouge × Altaica	3	1.563	202	79	281	1.453
Paris × Altaica	3	0.320	211	68	279	0.585
Asp. × Altaica	4	0.105	336	155	491	11.297***
Laur. × Altaica	1	—	69	21	90	0.133
Merv. × Altaica	3	5.142	213	91	304	3.947*
K. driv × Altaica	7	4.888	451	257	708	48.211***
Asiat × Altaica	1	—	68	26	94	0.355
Berliner × Altaica	3	0.501	299	154	453	19.550***
SL × Altaica	3	7.775*	71	23	94	0.014
			53	38	91	13.630***
			53	37	90	12.459***
R. rouge × Sc. M	3	0.957	139	68	207	6.804**
R. rouge × Sc. S. Afr.	1	—	79	24	103	0.016
Paris × Sc. L	3	6.107*	113	32	145	0.664
			60	26	86	1.256
			67	38	105	7.013**
Maik. × Sc. S. Afr.	6	6.213	367	200	567	31.916***
TT te te × Fl. 23	1	—	35	58	93	69.251***
Asp. × Fl. 23	1	—	139	121	260	64.328***
K. driv × Fl. 23	1	—	161	132	293	68.827***
Merv. × Fl. 23	3	1.391	152	144	296	88.288***
Asiat. × Fl. 23	3	0.682	157	125	282	56.175***
Berl. × Fl. 23	3	3.238	250	131	381	17.891***

TABLE 5 *cont.*

Non-lobed × lobed						
Cross	No. of fam.	χ^2 for heterogeneity between families	Leaf form		Total	χ^2 for 3 : 1
			lobed	non-lobed		
Berl. × Sc. S. Afr.	4	19.619***	132	65	197	6.716**
			47	19	66	0.505
			70	78	148	60.577***
			51	47	98	27.551***
Berl. × Sc. L	3	3.728	179	128	307	45.630***
Berl. × Sc. R	2	0.002	149	94	243	24.265***
R. Liebl. × Sc. I	5	12.146*	42	48	90	38.533***
			58	65	123	50.864***
			47	44	91	26.465***
			160	111	271	36.813***
			75	41	116	6.621**
Sc. H × Fl. 23	1	—	283	91	374	0.089
Sc. H × Sc. M	3	0.332	191	49	240	2.689
Sc. H × Sc. R	2	4.475	90	35	125	0.600
SL × Sc. L	2	1.047	189	63	252	0.000

χ^2 for heterogeneity between crosses: 1. All crosses: 237.838***
2. Homogeneous crosses: 197.531***

might possible have been classified as lobed in a segregating family. It should be mentioned in this connection that the non-lobed *serriola* form, Sc. H, occasionally produces leaves with very slight lobing.

Heterogeneity between families. — The eight crosses which are listed below show significant heterogeneity between F_2-families.

Cross	Hetero-geneity	DF	Parents ♀	Parents ♂	F_1-plants grown	F_2-plants grown
Oakl. × Merv.	6.760*	2	Same	Same	Same year	Same year
» × R. rouge	13.925***	2	»	»	» »	» »
Paris × Sc. L.	6.107*	2	»	»	» »	» »
Berl. × Sc. S. Afr.	19.619***	3	»	»	» »	Different years
R. Liebl. × Sc. I	12.146*	4	»	»	Different years	» »
SL × Altaica	7.775*	2	»	» ?	Same year	Same year
Altaica × Asp.	5.066*	1	»	» ?	» »	» »
R. rouge × Oakl.	13.278**	4	Diff.	Diff.	Different years	Different years

As mentioned in the introduction, heterogeneity between families of the same cross was encountered also by DURST in the cross Big Boston ×

TABLE 6. *F_2-results of crosses between lobed parents.*

Cross	No. of fam.	Leaf form	
		lobed	non-lobed
Fl. 23×Altaica	3	292	0
Sc. L×Altaica	3	424	0
Sc. S. Afr.×Altaica	3	258	0

lobed *serriola* and it appears to be a common occurrence in crosses between lobed and non-lobed lettuce. Heterogeneity of this kind is often caused by difficulties in classification. The question of errors in classification and their bearing on the F_2-ratios generally, will be discussed later in this paper on the basis of the breeding behaviour of F_3-progenies. For the present it is sufficient to say that the F_2-results have not given any cause to suspect serious difficulties in classification with the exception of the cross Altaica×Asp., one of the 8 crosses in which the χ^2 for heterogeneity between F_2-families is significant. In this cross 8 F_2-plants, classified as lobed, have given entirely non-lobed progeny.

Difficulties in classification may be the cause of heterogeneity also in

TABLE 7. *F_2-results of crosses between non-lobed parents.*

Cross	No. of fam.	Leaf form	
		lobed	non-lobed
Berl.×Sc. H	12	0	865
K. driv×Sc. H	6	0	377
Asp.×Sc. H	3	0	239
Paris×K driv	3	0	299
Berl.×K driv	6	0	691
Berl.×Asp.	3	0	283
K. driv×Asp.	3	0	265
K. driv×Laur.	2	0	161
K. driv×Merv.	1	0	46
Laur.×Merv.	3	0	216
Asp.×R. rouge	1	0	116
R. Liebl.×Asp.	7	0	30
Forcing×Asp.	4	0	157 (a few slightly lobed plants)
Berl.×SL	6	0	516
Berl.×SP	2	0	261
K. driv×SL	3	0	266
Asp.×SL	1	0	93

the cross SL×Altaica. No F_2-data are available for assessment of the accuracy of the classification, but one might suspect that the modifying genes which operate in the cross between SL and Berl. (see above) originate from the SL parent and that they have been segregating also in the cross SL×Altaica.

With regard to the remaining six crosses however, there is no reason to suspect difficulties in classification. This applies especially to the crosses between non-lobed and oakleaved, in which the segregation always has been clear cut. The heterogeneity in the crosses may be due to differences in the environment in which the F_1-plants or the F_2-families were grown or to genotypic differences between the F_1-plants, such as might result from different parentage or from heterozygosity of the parents. The details concerning parents, F_1-plants and F_2-families given above for the 8 crosses under consideration have been added with these possibilities in mind.

Normally, progeny from a certain F_1-plant was grown once only, and the possible effect of differences in the environment of the F_2-families is therefore confounded with the effect of different F_1-plants. In the case of the two crosses Berl.×Sc. S. Afr. and R. Liebl.×Sc. I, progenies from the same F_1-plants were, however, grown more than one year and the data for these families in Table 5 are totals for the different years. A comparison between the results from the different years has failed to reveal any significant differences, but the data are not sufficient to justify the conclusion that differences in the conditions under which the F_2-families have been grown are entirely without influence on the segregation ratios. Such influences, however, could not explain all the cases of heterogeneity under consideration as in five of the eight cases the F_2-families were grown the same year and side by side.

F_1-plants of the cross R. Liebl.×Sc. I, having both father and mother in common were grown for seed two different years. The effect of this on the F_2-results is shown by the following analysis, but it is impossible to decide whether this effect is due to differences in the environment of the F_1-plants or to genotypic differences between F_1-plants grown different years.

F_1-plants grown	χ^2 for heterogeneity between F_2-families	DF
Same year	1.612	3
Different years	10.534**	1
	12.146**	4

The varieties R. rouge and Oakl. were crossed on two separate oc-

casions and on each occasion F_1-plants were grown the same year and similarly the F_2-families. The χ^2-analysis gave the following results:

	χ^2	DF
Between families of the 1st. cross	0.839	1
» » » » 2nd. »	4.189	2
» the two crosses	8.250**	1
	13.278**	4

In this case the heterogeneity may have arisen because different parent plants were used, because the F_1-plants were grown in different environment or possibly because the F_2-families were grown different years. The data from the three crosses Oakl.×Merv., Oakl.×R. rouge and Paris×Sc. I, show that heterogeneity between F_2-families, grown under the same conditions, may arise even if the F_1-plants have the same parents and have been grown side by side.

It is difficult to imagine that the difference in environment of plants growing a few feet from each other and flowering at the same time could be sufficient to produce significant differences in the segregation ratios of the progenies. However, the flowering period of a lettuce plant normally lasts several weeks. It is conceivable that the weather conditions which prevail at the time of fertilisation can have a different influence on the efficiency of gametes of different kinds. If that is so heterogeneity could arise if the seed for the different F_2-families happened to come from flowers which had been fertilised at different times.

As the lettuce is almost completely autogamous it would be remarkable if the plants used for crossing were heterozygous for genes which in one way or another influence the segregation ratios between different leaf form types.

Unfortunately the data do not allow any definite conclusions to be drawn concerning the cause of the discrepancies and further speculation at this stage would be futile. The behaviour of the different leaf form types in F_2 obviously does not conform to any simple mendelian scheme. Some ratios agree with 3 : 1, 9 : 7 or 15 : 1, but inferences made from such data concerning the genotype of the parents are contradicted by results of other crosses. DURST's hypothesis of complementary genes is capable of explaining the results of some of the crosses, but, as will be shown below, evidence which proves that this hypothesis is untenable has been obtained from the two other sources, namely from crosses between non-lobed segregates and from crosses involving an anthocyanin character which is linked to the lobed leaf character.

3. Crosses between non-lobed segregates

One of the first crosses made in the course of this study was R. Liebl.×Sc. I. Apart from a significant heterogeneity between families the F_2-results of this cross were in good agreement with a 9 : 7 ratio (χ^2 for 9 : 7 = 0.263) and they appeared to be supported by data from the F_3-generation. Out of 16 F_2-families originating from lobed F_2-plants, 2 bred true for the lobed character, 5 segregated in proportions readily acceptable as 3 : 1 ratios, whereas the remaining 9 segregated with a more or less pronounced excess of non-lobed plants, the proportions varying from 29 : 16 to 21 : 22. The results did not give any serious reason for questioning DURST's hypothesis and crosses were made between non-lobed plants in F_2 and in F_3 with a view to isolating non-lobed lines homozygous for one or the other of the two dominant genes. The results of these crosses are presented in Table 8. They are rather

TABLE 8. *Test crosses between non-lobed segregates.*

Parents		Crosses		Number of authentic hybrids
Designation	Marker genes	Lobed plants	Non-lobed plants	
2025—15/42×23—1	$l \times T$	0	11	8
23—7×2025—4/42	$l \times T$	0	17	8
» × » 10/42	$l \times T$	0	46	21
» × » 40/42	$l \times T$	0	35	11
» × » 47/42	$l \times T$	0	46	19
» × » 50/42	$l \times T$	0	28	14
» ×23—1	$l \times T$	0	10	3

fragmentary because of practical difficulties in getting a material of the size of this F_3-generation sown early enough to come into flower before autumn dampness jeopardised the work of crossing.

' The two parents, 23—1 and 23—7, are non-lobed lines originating from non-lobed plants in F_2-families 3310/39 and 3314/39. These two families segregated as follows:

F_3 fam.	Lobed	Non-lobed	Total	χ^2 for 3:1	χ^2 for 9:7	P for 9:7
3310/39 (line 23—1)	37	8	45	1.252	12.335	< 0.001
3314/39 (» 23—7)	32	11	43	0.008	5.768	0.01—0.02

If the hypothesis of dihybrid segregation is accepted, both F_2-families must be regarded as segregating for one of the complementary genes only. Non-lobed plants from these families should therefore be homo-

zygous for one dominant and one recessive gene, *i. e.* they must be of the type *AAbb* or *aaBB*. As the hybrids between 23—1 and 23—7 were non-lobed (see last line in Table 8) they must both have the same genotype, say *AAbb*.

These two lines were crossed with a number of non-lobed F_2-plants chosen in such a way that successful crosses could be recognised by means of a dominant gene, *T*, for presence of anthocyanin. The purpose of the crosses was to identify F_2-plants with the genotype *aaBB* or *aaBb*. The former would give F_1-families consisting only of lobed plants, the latter would give lobed and non-lobed in equal proportions. Six of the crosses produced 8 or more authentic hybrids which were all non-lobed. The chances are very small that lobed individuals should fail to appear in a family of 8 plants when a 1:1 segregation is expected. $\left[\left(\frac{1}{2}\right)^8 = 0.004\right]$. It can therefore safely be assumed that none of the six F_2-plants under test had the hypothetical gene *B* in single or double dose. If DURST's complementary gene hypothesis is correct three out of seven F_2-plants would be expected to have the gene *B* and the chances that they should be missed in a test on six plants is $\left(\frac{4}{7}\right)^6 = 0.035$. A probability of only 3.5 % must be considered as strong evidence against the hypothesis and, although not conclusive, these test crosses threw very serious doubts on the existence of complementary genes for lobing.

4. Crosses involving the anthocyanin gene v

The lobed leaved form of *L. serriola* from South Africa was found to have a recessive gene which has the effect of gradually reducing the anthocyanin pigmentation as the plant develops. Seedlings of Sc. S. Afr. have perfectly normal anthocyanin colour, whereas the fully developed plants are almost devoid of any anthocyanin pigmentation. A fuller description of this gene, which has been designated *v*, will be given elsewhere. This gene segregated together with the genes for leaf form in four crosses, results of which are found in Table 9.

There are two very striking features about the data in this table. The first is that not a single non-lobed plant with the recessive anthocyanin character was recovered in any of the crosses. The second is the parallel behaviour of the segregation ratios lobed : non-lobed and *V* : *v*. In the first cross, Sc. S. Afr. × Sc. H, both ratios are in good agreement with 3 : 1, but in the other three crosses, where there is a large deficiency

TABLE 9. *Crosses involving the gene v.*

Crosses	Lobed		non-lobed		Total	χ^2 3 : 1	
	V	v	V	v		Lobed : non-lobed	V : v
Sc. S. Afr. × Sc. H	262	116	136	0	514	0.584	1.621
Maik. × Sc. S. Afr. (and reciprocal)	330	95	244	0	669	46.960***	41.615***
Berl. × Sc. S. Afr.	164	48	151	0	363	53.334***	26.851***
	Oakleaved		Lobed				
	V	v	V	v			
Oakl. × Sc. S. Afr. (and reciprocal)	218	0	0	20	238	34.963***	34.963***

of the lobed type, there is also a highly significant deficiency of *vv*-plants.

The result of the first cross, Sc. S. Afr. × Sc. H, shows that the two parents differ in only one pair of genes for leaf form and that the dominant allele is absolutely, or very closely, linked to the recessive anthocyanin gene *v*.

The ratio lobed : non-lobed in the next two crosses is intermediate between 3 : 1 and 9 : 7 and significantly different from both. The two crosses are in good agreement (the χ^2 for heterogeneity is only 2.617) and give together 637 lobed : 395 non-lobed. The χ^2 for deviation from 3 : 1 is 96.997*** and that from 9 : 7 is 7.386**. The segregation ratios in the crosses Maik. × Sc. S. Afr. and Berl. × Sc. S. Afr. are therefore not satisfactorily explained by the assumption that two complementary genes are involved and on this basis it is also impossible to explain the absence of non-lobed plants with the recessive anthocyanin character. As the linkage between the recessive anthocyanin gene and one dominant gene for lobing appears to be absolute, it follows that all plants recessive for the anthocyanin gene must be homozygous for this gene for lobing. Now, DURST's hypothesis implies that $^1/_4$ of all such plants should be non-lobed being recessive for the second, complementary, gene for lobing. The chance that a type with an expectation of $^1/_4$ should fail to appear among 143 plants is extremely small $\left[\left(\frac{3}{4}\right)^{143} = 1.36 \times 10^{-18}\right]$.

The results of the two crosses Maik. × Sc. S. Afr. and Berl. × Sc. S. Afr.

are therefore not capable of being explained according to DURST's hypothesis, nor do they support WHITAKER's suggestion that there is only one basic gene for lobing but that the 3 :1 ratio is disturbed by the segregation of genes affecting the serration of the leaf margin. They strongly suggest that only one locus for leaf form is involved and that the *v*-locus is absolutely linked to this locus. The F_2-segregation must therefore be considered as monohybrid with a large deficiency in the dominant, lobed class.

A similar deficiency of lobed plants is found in the last cross in Table 6, Oakl.×Sc. S. Afr. but here lobed is the recessive character. The ratio oakleaved : lobed is a very poor fit for 3 : 1. In fact it agrees quite well with 15 :1, the χ^2 for deviation from this ratio being only 1.883. (P=0.10—0.20), but the possibility that the character oakleaved should be determined by duplicate genes is ruled out by the non appearance of oakleaved plants with the recessive anthocyanin character. Such plants would be expected with a frequency of 3 in every 15 oakleaved and the probability that they should fail to appear among 218 individuals is obviously extremely small $\left[\left(\frac{4}{5}\right)^{218} = 7.5 \times 10^{-22}\right]$. It is therefore certain that only one pair of genes segregates also in this cross. The linkage with the anthocyanin gene shows that the same locus is involved here as in the crosses between Sc. S. Afr. and non-lobed forms. This proves beyond doubt that there are three alleles at this locus. Bearing in mind DURST's symbols U_1 and U_2, these alleles will be designated U_{oak}, U_{lob} and u. There is a deficiency of plants with the allele U_{lob} in all three crosses.

5. F_3-generations

Table 10 gives a summary of F_3-results from some of the crosses and Table 11 illustrates the distribution of families with different percentages of the recessive type. The proportions of non-lobed plants in F_2 of the respective crosses has also been indicated in Table 11.

The F_3-results support the F_2-data in as much as they show very bad agreement with the expectation on a basis of monohybrid inheritance. The ratios between true breeding and segregating families are usually poor fits for 1 : 2, but the number of families in the individual crosses are too small for the deviations to be significant. Even in the case of the ratio 22 : 25, observed in the cross Oakl.×Forcing, the deviation is barely significant at the 5 % level. An excess of segregating families

TABLE 10. *F_3-results.*

Families from F_2-plants with the dominant character								
Cross	Number of families				Totals for segr. fams.			
	true breeding dominant	segregating	true breeding recessive	Total	χ^2 for heterogeneity between families	dominant	recessive	χ^2 for 3 : 1
Oakl. × Altaica	3	10	0	13	15.253	369	95	5.069*
Oakl. × Forcing	22	25	0	47	56.449***			
TT tete × Oakl.	4	10	0	14	22.340**			
Oakl. × Sc. H	8	15	0	23	27.327*			
Oakl. × K. driv	6	15	0	21	28.866*			
K. driv × Eichenbl.	7	20	0	27	81.168***			
Asp. × Altaica	3	19	8	30	55.269***			
R. Liebl. × Sc. I	2	14	0	16	33.828**			
Berl. × Sc. R	6	25	1	32	62.799***			
Berl. × Fl. 23	9	23	0	32	73.968***			
K. driv × Fl. 23	8	23	0	31	168.894***			
Sc. H × Fl. 23	8	14	0	22	21.269	457	89	22.039***
R. rouge × Sc. S. Afr.	18	23	0	41	12.484	777	224	3.671

Families from F_2-plants with the recessive character				
Cross	Number of families			
	true breeding dominant	segregating	true breeding recessive	Total
Oakl.×Altaica	0	0	4	4
Oakl.×Forcing	0	0	4	4
TT tete×Oakl.	0	0	6	6
Oakl.×Sc. H	0	0	6	6
Asp.×Altaica	0	0	6	6
R. Liebl.×Sc. I	0	0	12	12
Berl.×Sc. R	0	1	15	16
K. driv×Fl. 23	0	1	19	20
Sc. H×Fl. 23	0	0	2	2
R. rouge× Sc. S. Afr.	0	0	6	6

usually corresponds to an excess of the recessive type in F_2 and vice versa. There is a very wide range with regard to the percentage of non-lobed plants. This is no doubt partly due to the fact that the families

TABLE 11. *Segregating F_3-families.*

Cross	Number of families in classes according to percentage of the recessive type															Total
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	
					F_2											
Oakl.×Altaica		1	1	2	4	1	0	1								10
					F_2											
Oakl.×Forcing	1	1	2	5	4	4	4	4								25
				F_2												
TT te te×Oakl.		1	3	2	2	1	0	1								10
					F_2											
Oakl.×Sc. H	(2)		1	1	1	5	1	2	1	1						13
							F_2									
Oakl.×K. driv					2	6	3	3	1							15
							F_2									
K. driv×Eichenbl.				3	3	5	5	3	1							20
							F_2									
Asp.×Altaica		1	2	1	1	2	1	3	2	1	3	0	0	2		19
					F_2											
R. rouge×Sc. S. Afr.		2	3	5	6	2	3	1	1							23
					F_2											
Sc. H×Fl. 23	1	2	3	2	4	2										14
									F_2							
K. driv×Fl. 23		1	0	4	1	6	4	0	2	2	1	2				23
											F_2					
R. Liebl.×Sc. I				2	0	3	0	3	1	3	2					14
								F_2								
Berl.×Sc. R				4	1	5	6	3	3	3	1					26
							F_2									
Berl.×Fl. 23				1	0	4	3	7	5	1	2					23

often are very small, but the significant χ^2's for heterogeneity between segregating families in Table 10 show that real differences exist. A certain correlation between the proportions of non-lobed plants in the F_3-generation and the distribution of the F_3-families is apparent.

The χ^2 for heterogeneity between segregating families is significant for all the crosses except Oakl.×Altaica, Sc. H×Fl. 23 and R. rouge× Sc. S. Afr. In all these three crosses the totals show a deficiency of the recessive type, although this deficiency does not quite reach the 5 % level of significance in the cross R. rouge×Sc. S. Afr. The F_3-families of the cross Sc. H×Fl. 23 were exposed to a severe attack of *Bremia* and many plants were lost before they could be classified. The ratio between lobed and non-lobed in F_2 of this cross was normal and it is

possible that there is a connection between the deficiency of non-lobed plants in F_3 and the incidence of the disease.

With a few exceptions to be considered later, progenies of F_2-plants with the recessive character have bred true for this character.

IV. DISCUSSION

The results of the crosses in which the allelic pair *V—v* segregates leave no doubt that the leaf form characters oakleaved, lobed and non-lobed are determined by three multiple alleles. The abnormal F_2-segregations must therefore be interpreted as disturbed monohybrid ratios.

The crosses between diffferent lobed forms, presented in Table 6, prove that Sc. S. Afr. does not differ from Sc. L, Fl. 23 or Altaica with regard to genes for the lobed character. In view of the very similar behaviour of the other lobed forms in various crosses it is reasonable to conclude that all lobed forms have the allele U_{lob}.

Although several of the crosses between lobed and non-lobed forms give F_2-segregations in good agreement with a 9 : 7 ratio, many are intermediate between this ratio and 3 : 1, and significantly different from both. This evidence alone makes it difficult to accept DURST's hypothesis of complementary genes for lobing and the test crosses between lobed segregates in R. Liebl.×Sc. I has failed to prove the existence of such genes. It is therefore safe to generalise the conclusion from the crosses with Sc. S. Afr. to the effect that the three leaf form types in lettuce are controlled by three alleles at the *U*-locus.

It remains to consider the cause of the drastic disturbances observed in F_2 and F_3 of many of the crosses. It was pointed out when considering the F_2-results that the abnormalities must be genetically determined as certain parents consistently give more abnormal ratios than others. Disturbed segregations may be due to incomplete manifestation of genes, to differential elimination of zygotes or to the fact that gametes carrying one allele function less frequently than gametes carrying another. These three possibilities will be considered in the light of the available data.

Incomplete manifestation. — The data in Table 10 show that the classification of the F_2-plants has on the whole been successful, but some cases of mis-classification are revealed. In the two crosses Berl.× Sc. R. and especially Asp.×Altaica some F_2-plants classified as lobed were genotypically non-lobed. This may be due to the presence of modifying genes as in the cross Berl.×SL (see p. 359). These errors,

which have the effect of increasing the number in the lobed class at the expense of non-lobed, cannot have been the cause of the abnormal F_2-ratios. Classification errors in the opposite direction, caused by incomplete manifestation of the dominant character could, however, have such an effect.

This possibility need not be considered with regard to the oakleaved type, partly because the deviations from 3 : 1 are in some cases towards an excess, in others towards a deficiency of plants with this character, and partly because segregation is always clear cut in families where this character is involved. The manifestation of the lobed character, on the other hand, is much less definite and classification in segregating families is sometimes difficult. This, together with the fact that there so often is a deficiency of this type, but never an excess, makes it necessary to consider whether mis-classification of lobed plants can explain the disturbed F_2-ratios. Data for such tests are available only for the two crosses Berl.×Sc. R. and K. driv×Fl. 23, in each of which one F_2-plant, classified as non-lobed, has been shown by the F_3-results to be heterozygous. The method quoted by MATHER (1938, p. 84) has been used below to ascertain whether such a small proportion of errors in classification can account for the large deviations from a 3 : 1 ratio in F_2 of the two crosses.

Berl.×Sc. R.

F_2	Observed	Expected	$\frac{(a-nm)^2}{nm}$
lobed	149	157.1355	0.4212
non-lobed	94	85.8645	0.7708
Total	243	243.0000	
F_3			
lobed	1	4.6798	2.8935
non-lobed	15	11.3202	1.1962
Total	16	16.0000	5.2817*

K. driv×Fl. 23.

F_2			
lobed	161	171.2210	0.6101
non-lobed	132	121.7790	0.8579
Total	293	293.0000	
F_3			
lobed	1	7.9700	6.0955
non-lobed	19	12.0300	4.0383
Total	20	20.0000	11.6018***

The χ^2 test shows serious lack of agreement between F_2 and F_3, especially in the cross K. driv×Fl. 23. This indicates that incomplete manifestation is unlikely to be the cause of the abnormal F_2-segregations. In the crosses between Sc. S. Afr. and non-lobed forms it is possible to identify plants homozygous for U_{lol}, because they are also homozygous for the recessive anthocyanin gene, *v*. The data from the first three crosses in Table 9 can therefore be written as follows:

	$U_{lob}U_{lob}$	$U_{lob}u$	uu	Lobed : non-lobed χ^2 for 3:1	$U_{lob}u$: uu χ^2 for 2:1
Sc. S. Afr.×Sc. H	116	262	136	0.584	1.190
Maik.×Sc. S. Afr.	95	330	244	46.960***	23.059***
Berl.×Sc. S. Afr.	48	164	151	53.334***	10.906***

Not only do the last two crosses suffer from an overall deficiency of lobed plants, but the ratio between homozygotes and heterozygotes in the lobed class also differs very significantly from the expected 1 : 2. There can be little doubt that the two anomalies have the same cause and no hypothesis is satisfactory unless it explains both.

The hypothesis of incomplete manifestation would mean that among the plants classified as non-lobed there is a considerable number which are genotypically lobed. Such plants must all be heterozygous, $U_{lob}u$, otherwise they would also have the recessive anthocyanin character and therefore be easily recognisable. An explanation on this basis would therefore not help to explain the aberrant ratio between homozygotes and heterozygotes, on the contrary it would imply that the ratio was even more abnormal. The conclusions drawn from the breeding results of non-lobed F_2-plants of the crosses Berl.×Sc. R and K. driv×Fl. 23 are therefore supported by this evidence, namely that the abnormal F_2-segregations are not capable of being explained by incomplete manifestation of the gene U_{lob}.

Elimination of zygotes. — Losses of zygotes in the seedling stage on a scale sufficient to explain such large deficiencies as are being considered here, could hardly escape notice. No abnormal seedling mortality has however been observed.

Elimination of zygotes before emergence would be more difficult to detect. However, in the two crosses of which details are given below, the deficiency, in this case of lobed plants, is so large that it remains significant even after adding to the lobed class the whole difference between number of seeds sown and number of plants classified.

Cross	Fam.	No. of seeds	Classified plants lobed	Classified plants non-lobed	Adjusted ratio lobed	Adjusted ratio non-lobed	χ^2 for 3:1
Merv.×Fl. 23	2160/48	150	44	49	101	49	
» × » »	2161/48	150	52	41	109	41	
» × » »	2162/48	150	56	54	96	54	
				Total	306	144	11.760***
K. driv×Fl. 23	2071/44	400	161	132	268	132	13.760***

It is, to say the least, highly improbable that all the unclassified zygotes should have been of the lobed type, but even this generous correction fails to make up for the deficiency in the dominant class. The disturbed ratios in these two crosses can therefore not be explained as due to elimination of zygotes before or after emergence and this is presumably true also of other crosses.

Reduced seed fertility, such as would be expected if zygotes were eliminated before the ripening of the seed, has been observed in several crosses between *serriola* and *sativa* forms, but this phenomenon is not correlated with the F_2-ratios between lobed and non-lobed. The crosses Berl.×Sc. S. Afr. and Maik.×Sc. S. Afr. serve to illustrate this point. Reduced seed setting was observed in the Maikönig cross, but not in the Berliner cross as shown by the following data:

Cross	Year	No. of plants	% of good seeds
Berl.×Sc. S. Afr.	1953	3	90.8
			93.8
			94.5
Berl.×Sc. S. Afr.	1955	3	91.9
			95.4
			88.7
Maik.×Sc. S. Afr.	1953	3	78.9
			76.1
			81.9
Sc. S. Afr.×Maik.	1953	3	63.1
			75.5
			80.0
Maik.×Sc. S. Afr.	1955	3	73.8
			70.1
			71.3

In spite of the superior seed setting, the first cross produced the same disturbed F_2-ratios as the second. As deficiency of lobed plants in F_2 is not always associated with reduced seed fertility of the F_1-generation,

it is improbable that elimination of lobed zygotes at an early embryonic stage can be the cause of the abnormal F_2-ratios.

A rather complicated hypothesis involving linkage between the *U*-locus and one or more complementary recessive genes with a lethal or sublethal effect would be necessary to explain the deviations from 3 : 1 on the basis of zygotic elimination. In order to explain the ratios between homozygotes and heterozygotes an even more complicated hypothesis would be required. Elimination of zygotes is therefore an unlikely explanation and it is not supported by any evidence.

Abnormal gametic ratios. — If the disturbed segregations are due to abnormal gametic ratios a certain numerical relationship must be expected between homozygous dominants, heterozygotes and homozygous recessives.

The two crosses Berl.×Sc. S.Afr. and Maik.×Sc. S. Afr. afford an opportunity of testing this relationship as the homozygous lobed plants can be identified by means of the recessive anthocyanin character.

The result of the test is presented below. The proportion of gametes carrying U_{lob} which have contributed to the F_2-generation has been calculated by taking the square root of the proportion of *vv* plants. If the proportion of such gametes is x, then the expected proportions in the three classes must be x^2, $2x(1-x)$ and $(1-x)^2$ respectively.

	Expected	Observed	$\frac{(a-nm)^2}{nm}$
$U_{lob.}U_{lob.}$	143.0	143	0.000
$U_{lob.}u$	482.3	494	0.277
uu	406.7	395	0.347
Total	1032.0	1032	0.624

The two crosses have been treated as one because of good agreement both in the ratio lobed : non-lobed and in the proportions between homozygotes and heterozygotes (heterogeneity χ^2's 2.617 and 0.007 respectively). It has been assumed that the gametic ratios are the same on the male and female side. The agreement between observed and expected numbers is very satisfactory ($\chi^2=0.624$; P=0.3—0.5). This strongly supports the assumption that the cause of the aberrant ratios is to be found in some abnormality on the gametic side.

With regard to the last cross in Table 9, Oakl.×Sc. S. Afr., the gametic ratio corresponding to the F_2-data is significantly different from that found in the previous two crosses. This has been proved by estimating, with the aid of the maximum likelihood method, the value

which best fits the whole body of data and testing the agreement between observed and expected values. The χ^2 for agreement was found to be 19.619***, but this result is not surprising in view of the frequent occurrence of heterogeneity between crosses as demonstrated earlier.

Some genetically controlled abnormality at gametogenesis or at fertilisation is suggested by the available evidence as the most likely explanation of the disturbed segregations of the alleles at the *U*-locus. With the data available at present it is not possible to decide whether it is a question of differential elimination of gametes or whether there are differences in fertilising capacity between gametes with different alleles. There are no weaknesses consistently associated with certain alleles. The allele U_{oak}, for instance, is sometimes at a disadvantage compared with *u*, but sometimes the opposite applies. Moreover, the allele U_{lob} is as a rule at a disadvantage compared with both the others, but in several crosses it shows normal segregation. It is probable that genes at other loci determine the ratios in which the alleles at the *U*-locus function at fertilisation. The heterogeneity between F_2-families observed in many crosses can be explained as due to segregation of such genes.

Experiments are in progress which are expected to throw further light on this question.

SUMMARY

The three leaf form types in lettuce, oakleaved, lobed and non-lobed are determined by the multiple alleles U_{oak}, U_{lob} and *u*.

The segregation of leaf form characters is frequently disturbed. There is sometimes an excess of the oakleaved type over the proportion expected on the basis of monohybrid inheritance, sometimes a deficiency. There is very often deficiency of the lobed type, but in no case has an excess of this type been found.

One line of *L. serriola* was found to have a recessive anthocyanin reducing gene which is absolutely or extremely closely linked to the *U*-locus. With the aid of this linkage it has been proved that DURST's hypothesis of complementary genes for the lobed character is untenable. This conclusion is supported by the fact that it has not been possible to produce a lobed F_1 by crossing non-lobed plants from F_2 and F_3 of segregating crosses.

It is suggested that genetically controlled abnormalities at gametogenesis or at fertilisation are the cause of the disturbed segregations.

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SELECTION PRESSURE ON IRRADIATED POPULATIONS OF DROSOPHILA MELANOGASTER

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I. INTRODUCTION

OUR knowledge concerning the influence of differences in social levels on the effects of ionizing radiation on Man is practically non-existent. This fact was the reason for starting the present experiments. It was, thus, tried to construct an experiment with *D. melanogaster*, which could serve as a model. As, however, it also is of a more general importance to study how selection pressure interacts with effects of irradiation, this article will be confined to these general problems whereas questions concerning the hazards within human societies will not be dealt with here.

There are obviously a very large number of problems which are of interest within this field, but, to make any investigation feasible, it is necessary to concentrate on a selected group among these problems. The important results of the pioneer work of WALLACE (1953, 1954, 1956) have been of a great value for us when planning our own experiments. These plans were made up in the beginning of 1956, and the experiments started (as described below) after some months of preparations in Dec. 1956. To study the changes which by irradiations were induced in the populations, different kinds of tests have been undertaken. However, with gaining more experience, it was found that some of these did not give much information; they were consequently substituted by new kinds of tests.

It should be stressed that these experiments are kept as a kind of routine work in our laboratory. They are still going on, and the results which are presented below must therefore be looked upon as preliminary.

II. EXPERIMENTAL TECHNIQUE

The easiest way to describe the nature of our problems seems to be to give a short account of the working routine. The manual work is made in laboratory rooms at room temperature. Otherwise all cages and vials are kept in an incubator room at 25° C.

With regard to the breeding of our populations three different types may be distinguished; we name these types P, bb, and R. The management of one generation of the P-populations is shown diagrammatically in Fig. 1. After eclosion all flies of the population are, without etherization, irradiated with x-rays. The dose was 2000 r in the two first generations of population P 25 and in the first generation of population P 200 (concerning the definition of P 25 and P 200, see below); in all other irradiations the dose is 1500 r. When irradiating, the flies are kept in a special »irradiation chamber» (constructed by Dr. Lüning) which, in the direction towards the x-ray beam, has a window of leucite (Fig. 1 a). The irradiation is done by a Skandia Intensive apparatus operated at 15 ma, 170 kv with an inherent filtration corresponding to 3 mm Al. After irradiation the flies are shaken into cages (Fig. 1 b). These are ordinary boxes, sold in furnishing stores, for use in refrigerators. The lids of the boxes are fastened by taping. Their dimensions, in cm, are about $20 \times 10 \times 7$. In the lids — which are used as the bottoms of the boxes — two circular holes are made into which Petri dishes for the food are inserted from the outside. This way of inserting makes the change of Petri dishes very easy. The dishes are held tightly against the cage by a piece of foam rubber. The food consists of ordinary agar-cornmeal. When the Petri dishes are used for egg counts or collection of larvae they contain agar substance blackened by charcoal. For pouring the flies out of the boxes there is made, in one of the upper corners, a hole covered with a piece of gauze fastened with tape. The number of flies is not counted but each cage contains between 150 and 200 females and perhaps a somewhat lower number of males. That means that there are several cages belonging to each of the separate populations: 7—9 cages for P 25, the same number for P 200 and 8—10 for P 400.

At 5 p.m. two days after irradiation new Petri dishes, containing food blackened by charcoal, are inserted in place of the old dishes. The new dishes are removed the following morning at 9 a.m. i.e. after 16 hours of oviposition, and put for incubating for 28 more hours. Thus, at about 1 p.m. the next day, freshly hatched larvae are collected from

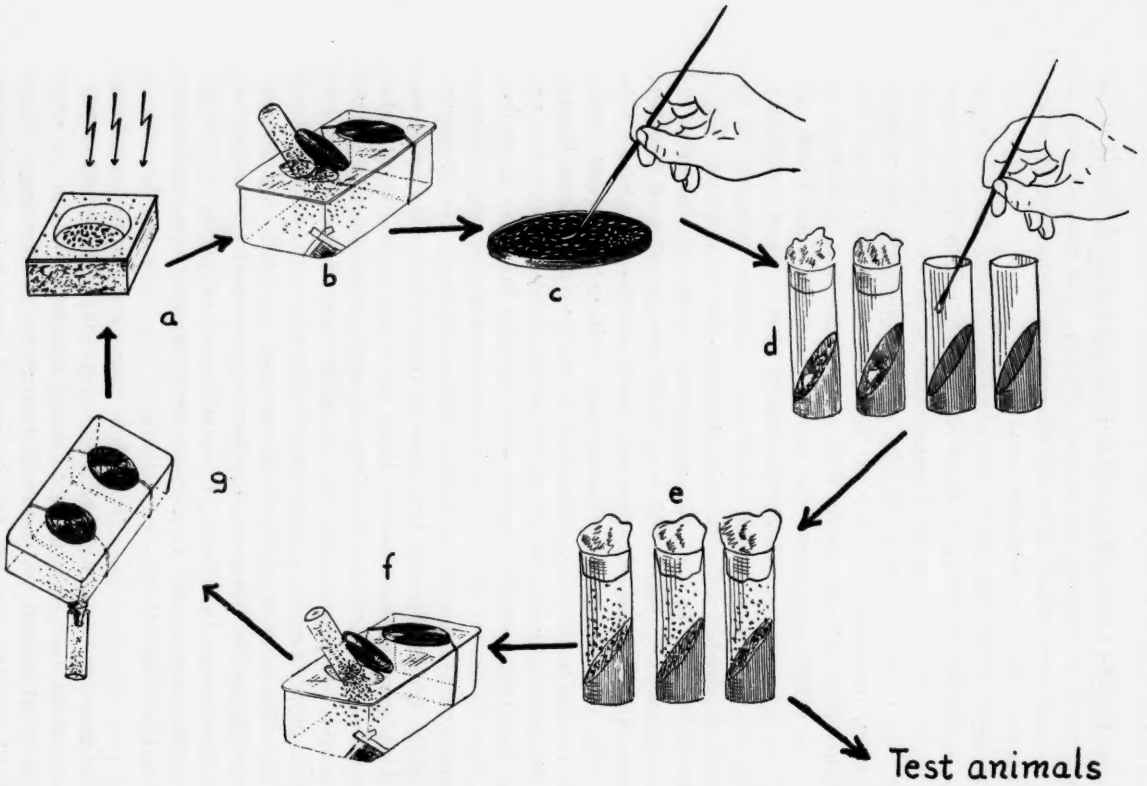

Fig. 1. Diagram showing the routine work in one generation (3 weeks) within the P-populations (see text for more details). — a, Irradiation in an »irradiation chamber». b, Transfer of irradiated flies to a population cage. c, Collection of freshly hatched larvae to vials with food; in population P 25 there are transferred 25 larvae to each vial and the corresponding number of larvae in populations P 200 and P 400 is 200 and 400 respectively. The larvae develop in these vials. e, Emergence of adult flies. From these test animals are collected and the rest is transferred to f, a population cage. g, The flies are shaken through a funnel into an empty vial for transfer to the irradiation chamber a.

the Petri dishes. The collection is made under a dissecting microscope with a fine brush (Fig. 1 c); from there the larvae are transferred to vials with ordinary food (Fig. 1 d). Different numbers of larvae are put into each vial within the different populations, 25 larvae in the vials of population P 25; 200 larvae in those of population P 200; and 400 larvae in those of population P 400. The inner dimensions of the vials are 27×90 mm. The amount of food is not weighed or measured. On the average each vial contains 12 ml of food and we believe that, from the point of view of the results, the variation in amount is quite insignificant, and, especially with regard to the large number of vials, that the small differences must cancel each other. Usually between 5000 and 6000 larvae are collected in this way for each generation. In several cases it has been impossible to get enough larvae from one single 16 hour period of oviposition. Therefore, the same procedure with oviposition from 5 p.m. to 9 a.m. is, if necessary, repeated for one or two days. After that the flies are discarded and the cages are put for cleaning. When the adults emerge in the vials (Fig. 1 e) they are etherized for inspection to ascertain against contaminations. Then they are transferred to cages (Fig. 1 f). The flies are collected daily until 16 days from the first day of oviposition within the parental generation. During this time the Petri dishes of the cages are changed every second day. Exactly three weeks after the irradiation of their parental generation the new flies are poured through a funnel to an empty vial (Fig. 1 g) and from there into the »irradiation chamber» for irradiation (Fig. 1 a). One complete generation thus takes exactly three weeks. To this description the following remark should be added. The facts that 1. each population is distributed to several cages; and, 2. that eclosion takes part in several more vials, with consequent matings before the flies are transferred to the cages, could seem to induce a certain amount of inbreeding. However, all flies from all cages belonging to the same population are mixed and irradiated together. We assume, therefore, that the tendencies to inbreeding, if any, are effectively counteracted by this procedure.

When preparing the present experiments Dr. H. J. MULLER visited our laboratory, and it was very valuable to get this opportunity to discuss the plans with him. He emphasized then that, in order to make comparisons with Man, it was of importance to check that the individual parents should get only a very limited offspring. To meet these demands, at least partly, we started the bb series. The diagram, Fig. 2, shows the different steps in the handling of these populations. Many

ched larvae to vials with food; in population P 25 there are transferred 25 larvae to each vial and the corresponding number of larvae in populations P 200 and P 400 is 200 and 400 respectively. The larvae develop in these vials. e, Emergence of adult flies. From these test animals are collected and the rest is transferred to f, a population cage. g, The flies are shaken through a funnel into an empty vial for transfer to the irradiation chamber a.

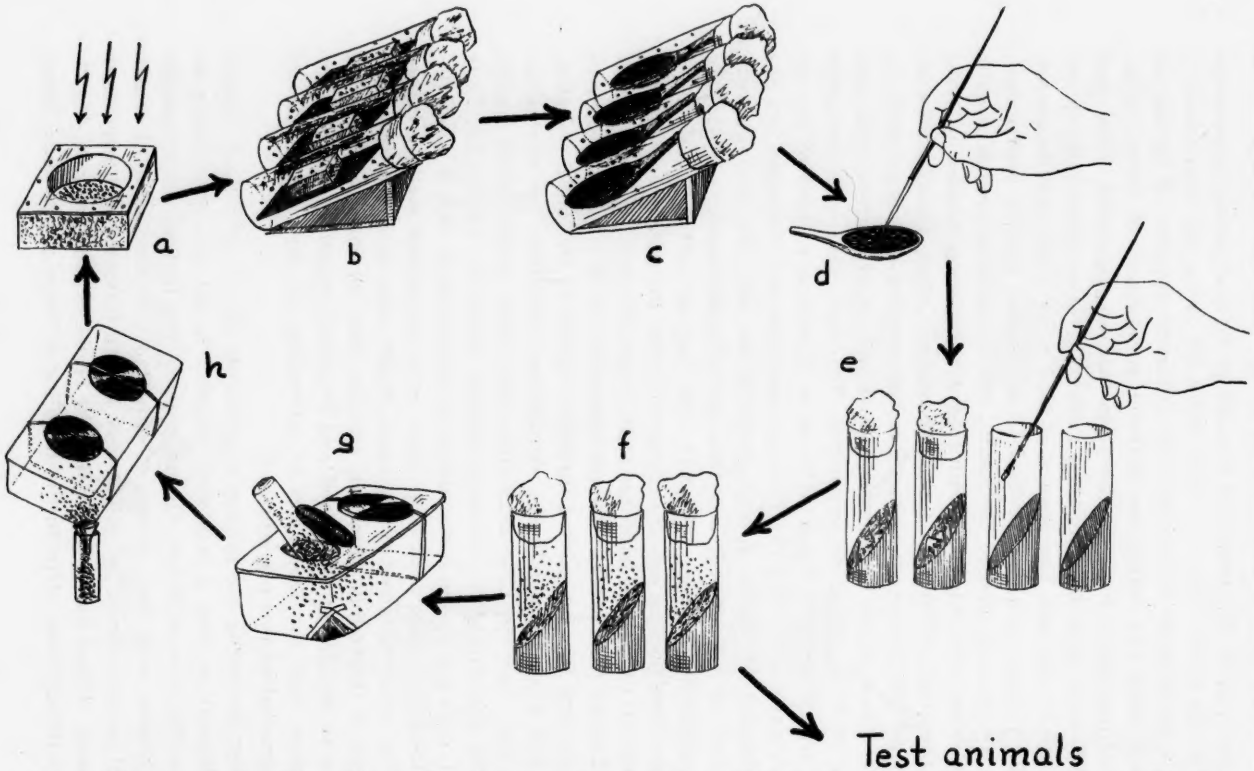

Fig. 2. Diagram showing the routine work in one generation 3 (weks) within the bb-populations. — a, Irradiation. b, The flies are, after etherization, transferred to vials, 5 females and some males into each vial. The food is given in pieces on cardboard slips. c, The card board slips with food are substituted by spoons with food. d, Freshly hatched larvae are collected from the spoons. A maximum number of 25 larvae is collected from each spoon, *i. e.* from the progeny of each group of 5 females. After this the bb-populations (bb 25 and bb 200) are treated as the P-populations (Fig. 1).

of the steps are similar to those of the P-populations. Immediately after irradiation (Fig. 2 a) the flies are etherized and transferred to empty vials: 5 females and some males into each vial. A card board slip with a piece of food is then placed into each vial (Fig. 2 b). Two days later, at 5 p.m. the card board slip with its piece of food is taken away and substituted by a small spoon with food blackened by charcoal (Fig. 1 c). The vials are numbered. Next morning at 9 a.m. — i.e., as in the P series, after 16 hours of oviposition — the spoons are transferred to new vials, each vial given the same number as that from which it is taken. The flies themselves remain in the empty vials, but get at 5 p.m. the same day, new spoons with blackened food. Meanwhile, the first set of spoons with their eggs are put for 28 hours of incubation and so, just as in the P-series, the collection of larvae begins at 1 p.m. (Fig. 1 d). When collecting the larvae, these are, as in the P-series, transferred into food containing vials (Fig. 2 e, f). 25 larvae are put into each vial of population bb 25 and 200 larvae into the vials of bb 200. The chief difference between the P- and the bb-series is that in the latter series no more than 25 larvae are ever collected from each separate group of 5 females. As it would be impossible to manage checks of virginity of these females, the number of paternal parents of the next generation is, however, unknown. (It should be mentioned that in the very first generation of bb 25 there were 6 to 8 females per vial and a maximum of 30 larvae were collected from each of these groups of 6 to 8 females.)

The R-population is kept in one single cage of dimensions about $30 \times 21 \times 13$ cm. It contains two Petri dishes with food. In contrast to the P- and bb-populations the dishes are placed inside the cage and no checks on selection pressure are made. After irradiation of all flies of the population the flies are transferred to the cage; four, or in some cases, five days after irradiation the flies are discarded and the Petri dishes transferred to a new cage. After eclosion of the next generation all flies are again irradiated and the same procedure is repeated. While the length of the generations in the P- and bb-populations is exactly three weeks, this length, in the case of the R-population, is exactly two weeks.

The lengths in time, as now described, of the intervals between the different steps in the management of the different populations, have been kept according to the plans through all generations. Only in a limited number of cases, in connection with holidays, illness and so forth, small and insignificant changes had to be done.

... the spoons. A maximum number of 25 larvae is collected from each spoon, *i. e.* from the progeny of each group of 5 females. After this the bb-populations (bb 25 and bb 200) are treated as the P-populations (Fig. 1).

III. ORIGIN AND START OF THE IRRADIATED POPULATIONS

a. Foundation stock

As one of the objects of the experiments was to try to follow the changes induced into the populations, it seemed most appropriate to start from an isogeneous and homozygous stock. For practical use the best inversion-linked dominant marker genes, in *D. melanogaster*, are situated in the second chromosome and, therefore, the decision was made to base the future chromosomal analyses on this chromosome (which also WALLACE has made in his studies). In the summer of 1956 there was, accordingly, produced a strain homozygous for chromosome 2. This strain emanated from a wild type stock of Swedish origin, called Örebro. Later, however, it seemed more desirable, to start the experiments from a stock which was isogeneous and homozygous for all of the three major chromosomes. New crosses were, therefore, made with flies from the strain which was already made homozygous for chromosome 2, and a strain, homozygous for the three chromosomes, and of a fairly good viability, was produced. This strain was, as described below, after some generations of propagation, used as the foundation stock for the irradiated populations P 25 and P 200.

It was the intention to use the foundation stock as a »control population» and it was named C—0. In accordance with the original plans, new homozygotisations — including only chromosomes 2 and 3 — should be done from time to time. In this way a control population C—1 was produced from C—0 and a control population C—2 from C—1.

b. The start of the irradiated populations P 25, P 200, bb 25 and bb 200

After having propagated the foundation stock C—0, part of it was irradiated on Dec. 8, 1956. This was the origin of population P 25. After further propagation of C—0 a new part of it was irradiated on Jan. 5, 1957 and this constituted the start of population P 200.

The population bb 25 was started in the following way. After having irradiated population P 25 on Jan. 19, 1957 larvae were first, according to the plans, collected Jan. 23 and 24 for the propagation of the population P 25 itself. The same irradiated flies were thereafter placed in vials with 6 to 8 females per vial (in all later generations, as described, 5 females per vial). From these vials larvae were collected on Jan. 26

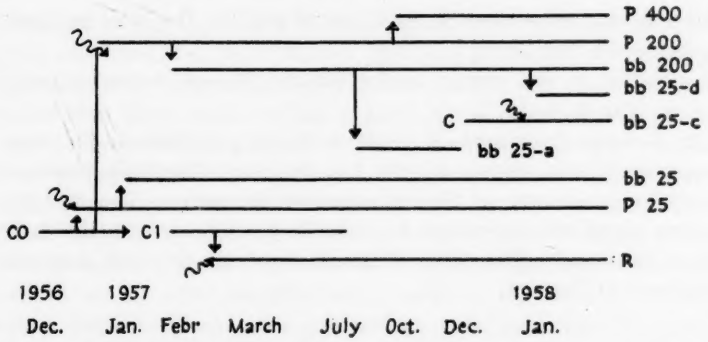

Fig. 3. Genealogy of the populations described in the present paper. — All populations with which investigations still are made are named in the right margin. From the Swedish wild type stock, »Örebro», the strain C0 was made homozygous and isogenous for the three major chromosomes number 1, 2, and 3. Except population bb 25—c all populations emanate from C0. From a wild type laboratory stock »Algeria», the strain C was made homozygous and isogenous for the three major chromosomes and the population bb 25—c emanates from this strain. Arrows show the starts of the different populations. The waved lines indicate the first irradiation performed within the different populations.

and 27, and this was the origin of bb 25. After the irradiation of P 200 on Febr. 16, 1957 collections of larvae for the propagation of the population P 200 itself were made on Febr. 20 and 21. The same irradiated flies were placed in vials with, as described, 5 females per vial, and

TABLE 1. *Dates of start.*

P 25 (first irradiation)	Dec. 8, 1956
P 200 (» »)	Jan. 5, 1957
bb 25 (first oviposition)	Jan. 24, 1957
bb 200 (» »)	Febr. 24, 1957
R (first irradiation)	March 26, 1957
bb 25—a (first oviposition)	July 23, 1957
P 400 (first oviposition)	Oct. 28, 1957
bb 25—c (first irradiation)	Jan. 27, 1958
bb 25—d (first oviposition)	Jan. 20, 1958

collections of larvae were made Febr. 26 and 27; this was the start of population bb 200.

Population R was started by irradiating flies of control population C—1 on March 26, 1957.

Fig. 3 shows the genealogy of the different populations. The start of those populations shown in the fig., but not described above, was caused by the results of the experiments themselves. The description of these starts will, therefore, be made in connection with the description of the results. The dates of the starts of the different populations are shown in Table 1.

IV. ANALYSES OF THE POPULATIONS

As indicated in the introduction different methods were tried for testing the changes induced into the populations. By gaining more experience it was found that some of the applied tests did not give much information and they were, accordingly, stopped or substituted by new methods. The methods, which in this way were abandoned, will be mentioned only briefly. Of other methods only the most important results will be given.

In the experiments of the present study the irradiations were, as described, given as acute x-ray doses. The reason for this was to some extent that our laboratory did not posses an equipment for chronic irradiation. The use of acute doses implies, however, the important possibility to analyze the nature of the flies which are not in themselves irradiated. Thus, all flies, intended for the analyses, were isolated from the population prior to its irradiation (concerning populations bb 25—c and bb 25—d, see however, below). The genome which these flies had inherited was, on the other hand, irradiated with the irradiation of their parents and their earlier ancestry. In the accounts given in the text and in the tables we are using the word »accumulated dose» for the sum of the x-ray doses which were given to the whole of this ancestry.

The purpose of analyzing the populations is: 1. to try to find out if the differences in management of the populations — with regard to selection pressure and to the control of the number of offspring per female — has had any influence when comparing the different populations at moment of equal (or nearly equal) accumulated doses; and 2. in the case such differences can be stated, to try to find that pathways through which these differences come into play.

The tests so far applied, may be grouped into two major categories. On the one hand, the performance and characteristics of the population individuals themselves were studied; while, on the other hand, analyses also were made on the nature of the chromosomes, if they include lethals, semilethals and so forth. Tests within these two categories will be described below, but it may already be said here that up to now (February 1958), no clear-cut differences between the different populations have been found. There is, however, another kind of tests, which, though not made intentionally for testing the populations, have shown to be the most sensitive ones; namely, the ease or difficulty which is met in managing the populations. It is, therefore, appropriate to begin the descriptions with this kind of questions.

a. Comparisons of the difficulties in handling the populations P 25, P 200, bb 25 and bb 200

The following remark may first be made. To keep the populations alive a certain number of larvae must be collected for each generation. It is, however, not the absolute number of larvae which is in itself relevant; what is of importance is that the number is large enough to keep the number of flies within the population on a sensibly unchanged level. It does not matter if this level is, say, 5000 or 10000: if the females of a certain generation are laying fewer eggs than the females of the preceding generation, or if the eggs hatch to a lower percent, then there will, in the next generation be produced fewer females. And if the same happenings with regard to egglaying and hatching are repeated in the new generation, a kind of a chain reaction may lead to the ultimate extinction of the population.

Now, the abilities of those who have to collect the larvae, may, of course also influence the total number of larvae which actually is collected on a certain day. For the carrying on of the present population studies the laboratory has, (besides the author U.-B. JONSSON) two assistants, who are very trained and competent for this job. The following may, however, though rather seldomly, happen: if the working plan includes the collection of, for instance, 6000 larvae, and if the first days collection (i.e. from the ovipositions of the first 16 hours) has produced, say 5600 larvae, then 400 larvae should be collected the next day. But if there are very much of other works which must be done this next day, it may happen that the resting 400 larvae never are collected.

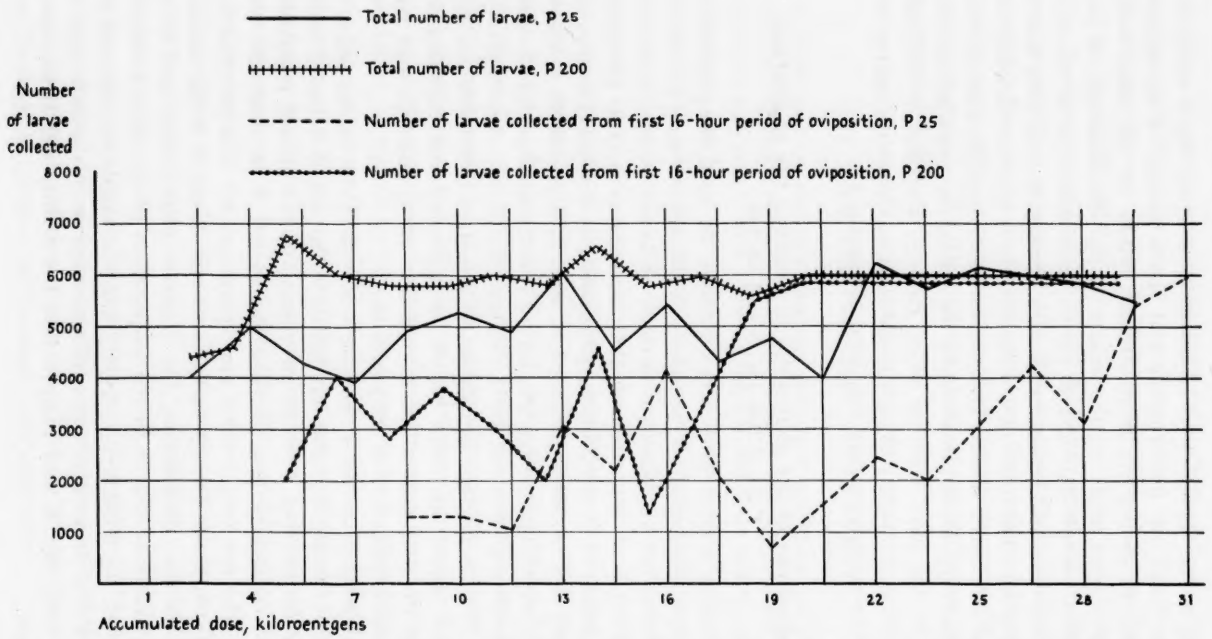

Fig. 4. Graphs showing the total number of larvae collected, at increasing accumulated doses, in populations P 25 and P 200; and the number of larvae collected within these populations at the first day of collection (*i. e.* after the first 16 hour period of ovipositions). The deep valley at 16 kiloroentgens in the number of larvae collected the first day in population P 200 is probably without significance. It happened on a hot day in July, and troubles are often met with in most cultures such days.

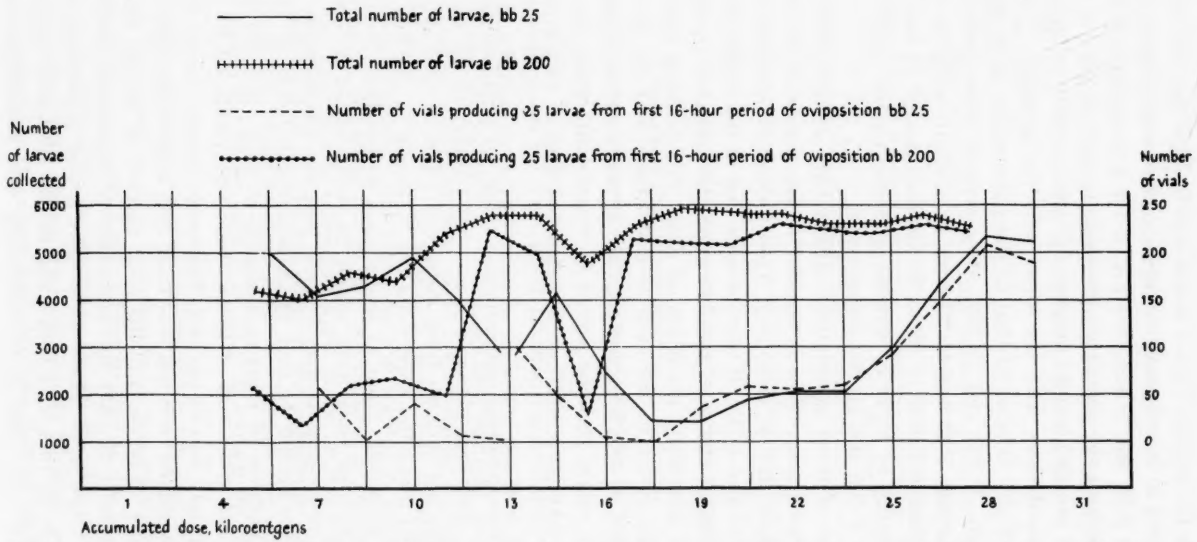

Fig. 5. Graphs showing the total number of larvae, collected at increasing accumulated doses, in populations bb 25 and bb 200; and the number of vials producing 25 larvae at the first day of collection (scale in the right margin). Concerning the deep valley in this number for population bb 200 at 16 kiloroentgens, see text to Fig. 4. Population bb 25 was close to extinction at 13 kiloroentgens; one genaration without irradiation was then intercalated.

Compare now the graphs Figs. 4 and 5 which show the total number of larvae collected at increasing amounts of accumulated doses, Fig. 4 for populations P 25 and P 200, and Fig. 5 for populations bb 25 and bb 200. In both cases the compared populations start at similar levels, but after a few generations the number of larvae which could be collected in the populations with the lower selection pressure, P 25 and bb 25, was markedly inferior to that of the populations with stronger selection pressure, P 200 and bb 200. After several more generations, however, populations P 25 and bb 25 grew in strength with the results that at an accumulated dose of 28 to 30 kiloroentgens P 25 and bb 25 had, from the point of view of easiness of handling the populations, caught up with P 200 and bb 200 respectively.

In the routine of these experiments efforts have always been made to collect as large a proportion as possible of the necessary number of larvae on the first day of collection. These numbers are also shown on Fig. 4 for P 25 and P 200. It may be seen that at the beginning only a limited fraction of the total number could be collected during the first day. With increasing accumulated doses this fraction increased, though markedly faster in the case of P 200 than in P 25. From an accumulated dose of 18.5 kiloroentgens all larvae were, in P 200, collected from one single 16 hour period of oviposition, while this happening did not take place until an accumulated dose of 29.5 kiloroentgens in P 25.

On Fig. 5 a corresponding comparison is made for populations bb 25 and bb 200. As described above, a maximum number of 25 larvae is collected in these populations from each vial (containing 5 females). The persons who make the collections try, however, to get this maximum number, 25 larvae, from as many vials as possible. The graph shows the numbers of vials from which 25 larvae could be collected the first day of collection. As may be seen this was considerably lower for bb 25 than for bb 200. In fact, at an accumulated dose of 13 kiloroentgens, the number of females available in bb 25 did not suffice for more than 164 vials, and of these, there was produced 25 larvae the first day in only one single vial. Moreover, though larvae on this occasion were collected over not less than four consecutive days, only 98 vials produced 25 larvae and the total number of larvae was only 2875. As it was believed that the extinction of population bb 25 was imminent, two steps were taken: 1. to start a new population of the bb 25 type but to do this with flies from bb 200 (this new population was named bb 25—a); and 2. to intercalate one generation without

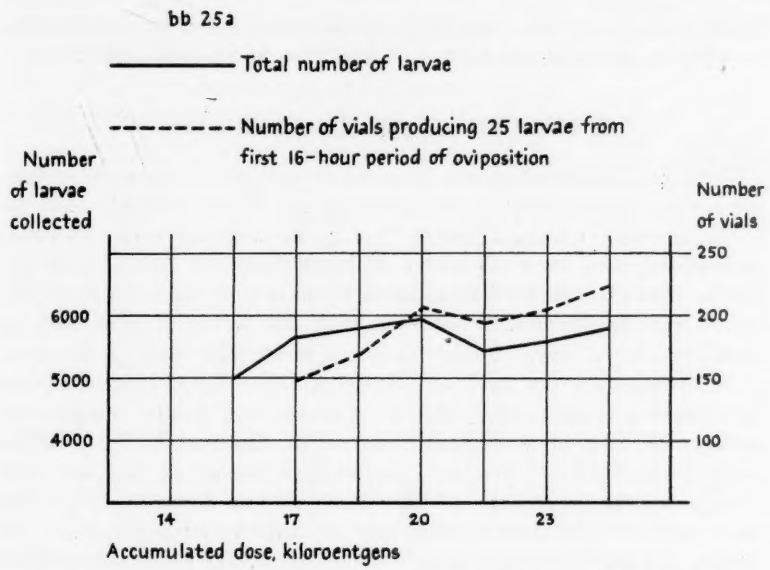

Fig. 6. Graphs for population bb 25—a, corresponding to the graphs, Fig. 5.

irradiation in bb 25. The effect of this intercalation was that in the next generation 25 larvae could be collected the first day in 100 vials. But during the following generations this number decreased again so that at an accumulated dose of 17.5 kiloroentgens, when the available number of females did not suffice to more than 59 vials, there was not a single vial which, after three days of collection, produced 25 larvae. Surprisingly, however, after this the number of vials with 25 larvae from the first day of collection grew more or less steadily and at an accumulated dose of 28 kiloroentgens the population bb 25 seems to be not much less vigorous than bb 200. Through the prosecution of the experiments it will be seen if bb 25 will remain on this high level of vigour.

The aim of starting population bb 25—a (derived from bb 200 at an accumulated dose of 15.5 kiloroentgens) was to study if its viability should decline to some level similar to that of bb 25. This was, however, never the case, its viability, shown by the proportion of vials which produced 25 larvae from one single 16 hour period of oviposition, remained on the same high level as that of bb 200 (Fig. 6). Po-

pulation bb 25—a was, therefore, discarded after 6 more generations, i.e. after an accumulated dose of 24.5 kiloroentgens (Dec. 1957).

b. Direct tests on population individuals

From the beginning it was planned to use larval competition tests for testing the viability of the populations. Hence, females were allowed to oviposit from 5 p.m. to 9 a.m. the next morning (16 hours oviposition), and after 28 hours of incubation 800 larvae were collected. These larvae were transferred to vials with ordinary food: 400 larvae were transferred to two vials with 200 larvae in each, and the same number of larvae were transferred to 16 vials with 25 larvae in each. From experience we knew (BONNIER 1956) that detrimental genes may cause a retardation of the development and it was thought that perhaps the rate of development should be different in the different populations. However, no such results were found; in fact we could confirm another experience of ours (BONNIER and JONSSON, 1957): that the rate of development is extremely sensitive to temperature. If one wishes to make a detailed study on developmental rates, observations at even short intervals, will not suffice if the development takes place in an incubator room where temperature differences as large as $\pm 1^\circ$ C are possible. For P 25 there were made such larval competition tests on 6 occasions and for populations P 200, bb 25, bb 200 on 5 occasions. As these tests did not give us much information of special value they were thereafter abandoned.

A further kind of test on population individuals was to compare the egglaying capacity and the hatchability of the eggs. At first, we used groups of five females, with usually two periods of 16 hours (from 5 p.m. to 9 a.m.) of oviposition. No checks were in these tests made on possible deaths of the females before the end of the tests. Later these tests were made on individual females and always after three consecutive 16-hour ovipositions, though without paying any attention to the virginity of the females.

From the beginning of making the egglaying tests on individual females, the following regulations were followed when computing the results. Females which died before the end of the third egglaying period, or which laid a total number of eggs less than 10 during the three periods, or which laid eggs none of which hatched, were excluded from the calculations. Furthermore, in computing the hatching percents, those females were also excluded which laid a total number of eggs

TABLE 2. *Analysis of egglaying capacities and egg-hatchability from individual females. All tests based on 3 consecutive ovipositions from 5 p.m. to 9 a.m. (16 hours).*

Population	Accumulated dose kiloroentgens	Eclosion e = early l = late	Number of females forming base of average of		Average number of eggs per female per hour of oviposition	Average of individual percents of hatched eggs	Percent females with 90.0 or higher percent of hatched eggs
			Number of eggs	Percent hatched eggs			
P 25	22	e	27	26	2.30 ± 0.15	78.1 ± 4.4	46
	22	l	13	13	2.52 ± 0.14	66.2 ± 7.1	23
	23.5	e	22	22	2.03 ± 0.13	68.5 ± 6.5	50
	23.5	l	24	21	1.68 ± 0.15	81.4 ± 3.4	33
	26.5		31	29	1.64 ± 0.11	79.9 ± 4.5	52
P 200	18.5		52	50	2.22 ± 0.11	81.3 ± 3.5	50
	20	e	28	27	2.39 ± 0.16	90.5 ± 2.8	74
	20	l	31	31	2.31 ± 0.12	83.0 ± 3.9	65
	23		30	29	1.74 ± 0.12	82.3 ± 4.0	55
P 400	21.5	e	29	28	1.60 ± 0.11	89.6 ± 2.6	71
	21.5	l	28	27	1.72 ± 0.15	82.2 ± 4.6	59
	23		10	10	1.52 ± 0.13	78.7 ± 7.9	50
bb 25	22	e	30	30	2.71 ± 0.19	87.2 ± 2.8	60
	22	l	19	16	1.39 ± 0.18	78.6 ± 4.3	19
	25		23	22	1.54 ± 0.11	80.5 ± 4.4	55
bb 200	18.5		34	33	1.72 ± 0.11	74.7 ± 3.5	30
	20	e	30	30	2.05 ± 0.10	79.1 ± 3.8	43
	20	l	27	27	2.08 ± 0.10	73.6 ± 4.9	33
	23		30	29	2.21 ± 0.17	72.4 ± 4.8	38
bb 25—a	20	e	32	32	2.34 ± 0.08	88.3 ± 2.6	56
	20	l	27	26	2.45 ± 0.15	74.4 ± 3.3	31

less than 30 during the three periods. Table 2 contains the results of the egg tests. In order to get some information of the selectional effect of growth rate, comparisons were made between egglaying capacities and egg hatchabilities of females which emerged »early» and »late» respectively. Early means in the case of P 25, bb 25, and bb 25—a, that the females had emerged not more than 9 days after the beginning of the oviposition (from which larvae were collected) of their mothers. In the case of P 200, P 400 and bb 200, early means that the corresponding number of days is 10. Table 2 shows the averages together

with their standard errors. In the case of hatchability this error does not, however, tell much of the degree of variation, as the distribution is very skew. Therefore, the table includes also a column showing the part of the observed hatching percents lying in the interval 90.0—100.0.

It may be shown (from unpublished data) that the variation of the hatchability percentages is to a rather high degree genetically determined. Table 2 shows now that, with one exception, this percent is higher for eggs from females which emerge early than from those which emerge late. However, even in the case of this exception — P 25 at an accumulated dose of 23.5 kiloroentgens — it may be seen, that the interval of hatching percents 90.0—100.0 includes a larger proportion of females among the early emerging females than among the late emerging ones.

From the beginning of studying egglaying and egg hatchability by pair matings, all females develop in vials with 25 larvae. Thus, in P 200, P 400, and bb 200, when after irradiation of the flies, larvae are collected and transferred to vials with 200, 400, and 200 larvae respectively, there are also produced a number of vials with 25 larvae each, and it is from flies emerging in these vials that the egglaying capacity and hatchability is observed. As this change in procedure was made only quite recently, it is too early to discuss any results.

Since quite recently, testing of the fertility of population males has

TABLE 3. *Frequency of sterile males.*

Population	Accumulated dose, kiloroentgens	Number of males tested (after exclusion of dead males)	Number sterile males
P 25	26.5	48	5
	29.5	48	6
bb 25	25	49	10
	28	49	12
P 200	24.5	45	3
	27.5	50	6
bb 200	24.5	49	3
P 400	24.5	47	7
	27.5	49	3
R	26	48	6
bb 25—a	24.5	47	3

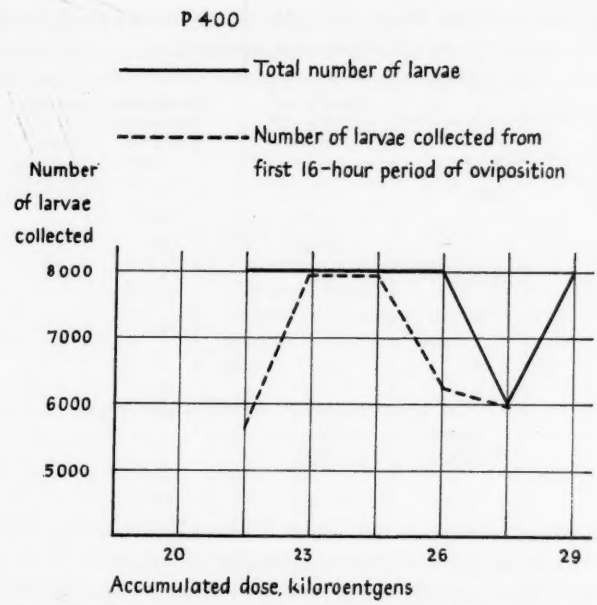

Fig. 7. Graphs for population P 400, corresponding to the graphs, Fig. 4.

been included among the routine tests. 50 males are taken out at random from the populations and are individually isolated in vials with ordinary food together with 3 virgin Cy L/Pm females from a stock which we know is of a good fertility. After four days the vials are inspected for presence of larvae. If no larvae are seen the males with their females are transferred to new vials with spoons containing food blackened by charcoal. There they remain for two days, and if still no larvae are seen the males are classified as sterile. It is unavoidable that now and then some males die before the test is ended; hence the table includes in most cases less than 50 males. With regard to the fact that this fertility test has been in work for only a very limited number of generations, no comparisons are possible (Table 3).

Because of the difficulties to state, by the direct tests applied, positive differences between the populations P 25 and P 200, population P 400 was started. It was derived from P 200 at an accumulated dose of 23 kiloroentgens (Fig. 7).

TABLE 4. *Accumulated doses at the three occasions (o—1, o—2, o—3) of chromosomal analysis.*

Population	Occasion of chromosomal analysis	Accumulated dose, kiloroentgens
P 25	o—1	7
	o—2	13
	o—3	22
bb 25	o—1	8.5
	o—2	13
	o—3	20.5
P 200	o—1	5
	o—2	12.5
	o—3	20
bb 200	o—1	5
	o—2	12.5
	o—3	20
bb 25—a	o—3	20
R	o—3	21

c. Analyses of chromosomes

Chromosomal analyses have, so far, been performed at three different occasions. They will below be referred to as o—1, o—2, o—3. Table 4 gives a summary of the accumulated doses at which these analyses were made. We have, as WALLACE, used the second chromosome and made the analyses according to the so-called Cy L-technique (WALLACE, 1956; BONNIER, 1957). Thus a number of males from the different populations are individually crossed with virgin Cy L/Pm females. From each of these crosses one single Cy L/+male is again crossed with virgin Cy L/Pm females, and among the progeny of this cross virgin Cy L/+females are crossed to Cy L/+brothers. This is made in vials, always with two females and one or two males per vial, and, if possible, two vials are started for each chromosome to be tested. The flies are moved to fresh vials after three days, when the old vials are discarded. The progeny is counted and classified twice: eight and eleven days after the discarding of their parents. We will name a strain produced from one, in this way isolated chromosome number 2 — whether propagated in homozygous condition or by mating Cy L/+ brothers and sisters — as a *chromosomal strain.*

TABLE 5. *Analysis of the number of Cy L/+flies in the progeny of homozygous and heterozygous crosses of Cy L/+ × Cy L/+.*

Population	Accumulated dose kiloroentgens	Average number of Cy L/+ flies	Source of variation	Degrees of freedom	Mean squares
P 25	7	85.75	P 25 v. bb 25	1	6326
bb 25	8.5	69.88	P 200 v. bb 200	1	1083
P 200	5	110.35	(P 25 + bb 25) v. (P 200 + bb 200)	1	50741
bb 200	5	103.93	Within populations		
			between chromosomal strains	102	1058
			between two vials of same strain	103	540

Significance of ratios 6326/1058 $0.025 > P > 0.01$
50741/1058 $P < 0.0005$
1058/540 $P = 0.0005$

As explained above (section 3 a) the homozygous foundation stock was also kept without any irradiation and as such used as a control stock C—0. It was planned to make, from time to time, new homozygotizations (only including chromosomes number 2 and 3). In this way control stock C—1 was derived from C—0. In o—1 and o—2 the progeny was also counted from crosses of the type +c/+c × Cy L/+P, where +c symbolizes a chromosome number 2 from population C—1 and +P a corresponding chromosome from the population under investigation. The results of these »control» crosses are, however, of less interest, and will, therefore, not be dealt with here.

When making the crosses for studying the survival rates in o—1 we observed that the total number of offspring was larger in populations P 200 and bb 200 than in populations P 25 and bb 25. As described above, these crosses consisted of crossing Cy L/+females to Cy L/+ males, where the wild type chromosomes of females and males were of common origin in the homozygous crosses, but — as described below — of different origin in the heterozygous crosses. These crosses were, in all populations, carried out in exactly the same manner (see description above). Even after exclusion of the wild type flies the remaining progeny, i.e. the Cy L/+flies, were larger in number in the populations P 200 and bb 200 than in populations P 25 and bb 25. Pooling the homozygous and heterozygous crosses (between which there could, on a priori grounds, be no differences in this respect,

which also could be stated statistically), Table 5 shows the means and the analysis of variance.

When trying to find an explanation it must be remembered that at o—1 the relative differences in the accumulated doses were rather marked between, on the one hand P 25 and bb 25, and on the other P 200 and bb 200; and that also the dose for bb 25, which showed the lowest average number of Cy L/+flies was, relatively seen, rather much higher than the dose for P 25. But whether the result is due to dose differences or to differences in selection pressure — which might influence egglaying capacity, egg hatchability, or just survival from oviposition to eclosion — it seems unavoidable to conclude pronounced dominance effects of the irradiated wild type chromosomes.

Any corresponding result was not found at o—2 or o—3.

As it seemed to be of great interest to study the spread of the mutant genes into the different populations we concentrated our investigations on the lethals in o—3. This was not because the lethals would in any way be more important to study than the less drastic mutations — the contrary is probably true — but because the difficulties to accomplish the investigation would be many times larger by including other mutants than the lethals. (When there are produced 3 per cent or less of wild type flies in a homozygous cross Cy L/+ × Cy L/+, the chromosomal strain is, by definition, looked upon as lethal.) We have, therefore, intercrossed all lethals in all possible directions. Every cross has not, in all cases, succeeded; Table 6 shows the total number of possible crosses and the number of successful crosses. To make it possible to carry through these studies the offspring from the heterozygous crosses Cy L/+ × Cy L/+ was not actually counted. After eclosion the vials were inspected from the outside by using special spectacles with a magnification of 3×. If several wild type flies were found, the cross was registered as a non lethal. If no, or only very few, wild type flies could be seen, the flies were etherized and counted.

When analyzing the results of the heterozygous crosses between different lethal strains, we have made two assumptions. 1. As the large majority of crosses were non lethal we assume that the very few unsuccessful crosses also were non lethal. 2. If a cross is non lethal, we suppose that the lethal genes, which make the intercrossed strains lethal, are non allelic. With methods now available the last of these assumptions can neither be proved nor disproved, and seems not necessarily to be true. It is, however, for the present discussion convenient to adhere to this assumption.

TABLE 6. *Analysis of lethal chromosomes.*

Population	Accumulated dose, kiloroentgens	Number of chromosomes tested in homozygous condition	Number of chromosomes lethal when homozygous	Percent lethal chromosomes	Number of possible heterozygous combinations of lethal chromosomes	Number of heterozygous crosses performed between lethal chromosomes	Number of lethal genes observed	Number of lethal loci observed	Spread index	Average number of lethal genes observed in lethal chromosomes
P 25	13	21	9	43	36	35	8	9	1.13	1.00
	22	52	29	56	406	406	22	30	1.36	1.03
P 200	12.5	18	6	33	15	14	5	6	1.20	1.00
	20	43	18	42	153	149	17	18	1.06	1.00
bb 25	13	24	6	25	15	12	6	6	1.00	1.00
	20.5	42	25	60	300	295	20	26	1.30	1.04
bb 200	12.5	25	11	44	36*	36*	5*	9*	1.80	1.00*
	20	52	32	62	496	496	24	36	1.50	1.13
bb 25—a	20	47	25	53	300	297	24	46	1.92	1.84
R	21	54	34	63	561	560	28	34	1.21	1.00

*) In bb 200 at 12.5 kr two of the eleven lethal strains were by mistake not included in the production of heterozygotes. The figures marked with an asterisk are thus computed on 23 instead of 25 tested chromosomes and on 9 instead of 11 lethal chromosomes.

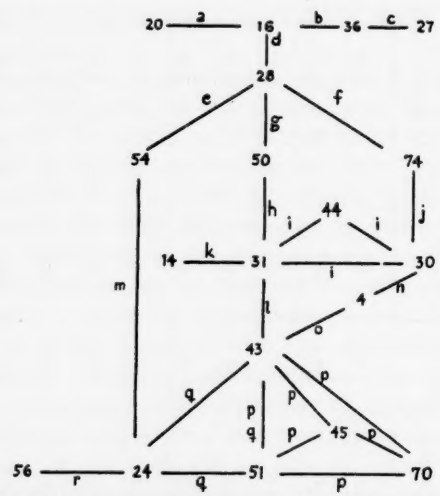

Fig. 8. The numbers shown in the figure are the record numbers of a part of the chromosomal strains analyzed at o—3 in population bb 25—a. Lines connecting two numbers indicate that the connected numbers are lethal when intercrossed. The letters are arbitrary gene-symbols.

Suppose now that we are considering three chromosomes which are lethal when homozygous. Giving them the record numbers 1, 2, 3 it may happen that all three crosses 1×2, 1×3, 2×3, are lethal. If so, there must be at least one common lethal gene (or allelic lethal genes) in each of the three chromosomes. It may also happen that 1×2 and 2×3 are lethal but not 1×3. In such a case we have observed at least two lethal genes, occupying altogether four loci, one in 1, two in 2, and one in 3. Fig. 8 shows a rather complicated case from population bb 25—a. The numbers are record numbers of lethal chromosomes, and those numbers which are connected by lines are lethal when intercrossed; the letters are arbitrary gene symbols. All combinations of strains 43, 45, 51, 70 are lethal, hence they have a common lethal gene, in the figure named *p*. Strain 24 crossed to 43 and 51 is also lethal but not when crossed to strains 45 or 70. There is thus another lethal gene, *q*, in the three chromosomes 24, 43 and 51. Going through all crosses in this way one may classify the observed lethal genes according to the number of chromosomes in which they (or allelic lethals) are found. If there are m_1 lethal genes, each observed in only one chromosome, m_2 lethal genes each observed in two chromosomes and so forth, then

the total number of observed lethal loci is obviously $S(im_i)$. Naming the total of observed lethal genes m, i.e. $m_1+m_2+\ldots=m$, the average number of chromosomes to which the individual lethal genes have spread, which we may call the *lethal spread index*, L, is obviously estimated by the equality.

$$L=\frac{S(im_i)}{m},$$

We may also go through our material by counting the number of chromosomes according to the number of observed lethal genes which they contain. Denoting by n_i the number of chromosomes with i observed lethals, the total number of observed lethal loci is obviously $S(in_i)$, hence, $S(im_i)$ and $S(in_i)$ must tally. If N_1 is the total number of lethal chromosomes and N the total of homozygously analyzed chromosomes, the two numbers

$$\frac{S(in_i)}{N_1} \text{ and } \frac{S(in_i)}{N}$$

show the average number of observed lethal genes per lethal chromosome respectively per population chromosome. Table 6 shows the results from o—2 and o—3. In some of the populations the spread index increased and in others it decreased from o—2 to o—3. The number of chromosomes analyzed was, however, small in o—2. By pooling the results from the populations P 25, P 200, bb 25 and bb 200 one finds a total of observed lethal genes and a total of lethal loci observed to be, at o—2, 24 and 30 and at o—3 83 and 110 respectively. The average spread index has thus increased from 1.25 to 1.33. This increment is, of course, not statistically significant, but may be taken as an indication.

When we are speaking of numbers of lethal genes per lethal chromosome, these numbers refer obviously to *observed* genes and are thus minimum numbers. It is possible that the lethal chromosomes may contain more lethal genes with a low spread index and therefore observed as one single gene.

d. Populations bb 25—c and bb 25—d

The results so far gained do not give any clear picture of what is happening within the populations. We may take, however, the following observations as *preliminary indications*: 1. The populations with

TABLE 7. *Comparisons of egg production and egg hatchability in populations bb 25—c and bb 25—d.*

Tests are made not only prior to irradiation (as in all other tests) but also after irradiation. The prior irradiation tests are made after pair matings with virgin females. Any check on virginity can not be made after irradiation. The first line in the table refers to the selection of the homozygous strain C (Fig. 3) from which bb 25—c was started after breeding it as a bb 25 population.

Population	Prior x-rays (p) after x-rays (a)	Accumulated dose kiloroentgens	Number of females		Average number of eggs per female per hour	Percent hatched
			Total	Available for egg counts		
bb 25—c	p	0	10	9	1.53	89.8
	p	0	44	42	1.38	79.2
bb 25—d	p	27.5	44	42	1.87	78.9
bb 25—c	a	1.5	44	34	0.86	34.4
bb 25—d	a	29.0	44	43	1.87	49.3

low selection pressure, P 25 and bb 25, were much more difficult to keep alive than those with a strong selection pressure, P 200 and bb 200; especially noteworthy is that bb 25 was close to extinction. 2. When the accumulated dose had reached a certain level, the viability of all populations seemed to increase with further increase of the accumulated dose. This process was, however, much slower in the populations with the low selection pressure than in those with the

TABLE 8. *Comparisons of number of vials with 25 larvae from the first 16 hour period of oviposition in populations bb 25—c and bb 25—d. These comparisons correspond to the graphs shown in Fig. 5.*

Population	Accumulated dose kiloroentgens	Number of vials				Total larvae
		With 25 larvae		With less than 25 larvae	Total	
		in 1 day	in 2 days			
bb 25—c	0	155	78	7	240	5975
	1.5	85	120	34	239	5675
bb 25—d	29.0	231		7	238	5900

strong pressure. 3. When transforming a population with strong pressure and good viability to a population with low pressure — the production of bb 25—a from bb 200 — no decrease in viability was observed.

It would be premature to formulate any hypothesis on these observations. We have, however, started two more populations, bb 25—c and bb 25—d (Table 1). During the last months of 1957 we did produce a new strain, C, homozygous for chromosomes number 1, 2 and 3. It was selected from a laboratory wild type stock from Algeria, thus of a quite different origin than the foundation stock C—0. With flies from this new homozygous strain, C, the population bb 25—c was started (Fig. 3), first by breeding it as a bb 25-population for one generation without irradiation, but thereafter with irradiations, just as in our other populations of the bb 25-type. The first irradiation was made on Jan. 27, 1958. Flies from bb 200, which had been irradiated on Jan. 25, 1958 (accumulated dose 29 kiloroentgens) were, parallel with this, bred according to the bb 25-type, with the first oviposition beginning Jan. 20, 1958. This population is named bb 25—d. It is our intention to observe these two populations, and to compare their fates somewhat more closely, at least for a limited number of generations. Tables 7 and 8 include the first observations from these populations.

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SUMMARY

(1) The present article describes the start and the first preliminary results of an experiment on the influence of selection pressure on irradiated populations of *Drosophila melanogaster*. It was planned as a model for investigating the influence of differences in social levels upon the effect of radiation on Man. As the problem of the interaction between selection pressure and radiation induced mutations also is of a more general interest from the point of view of radiation genetics and population genetics, the specific human aspect is, however, not dealt with here.

(2) All populations included in the experiment (except population bb 25—c, see below) emanate from a common foundation stock of non

irradiated wild type flies which, by ordinary methods, was made homozygous for the three chromosomes number 1, 2 and 3.

(3) The differences in strength of selection pressure between the different populations are checked and maintained by larval competition. While the fullgrown flies are kept in cages, their progeny, in the stage of freshly hatched larvae, are collected and transferred to vials with a standard amount of food (not measured but about 12 ml per vial) where they develop. In the populations named P 25, bb 25, bb 25—a, bb 25—c and bb 25—d, the number of larvae per vial is 25. Thus in all these populations there is a low selection pressure. In the populations named P 200 and bb 200, the corresponding number of larvae is 200 and in population P 400 the number is 400. In these populations the selection pressure is strong. The difference between the populations named P and bb respectively is that there is, in the former, no check on the number of offspring per female, whereas in populations bb the progeny of groups of five females never includes more than 25 flies. In the population named R there is neither any check of selection pressure nor on the number of offspring per female.

(4) After emergence of a new generation all flies are irradiated by an acute x-ray dose of 1.5 kiloroentgens. (In the two first generations of P 25 and the first generation of P 200 the dose was 2 kiloroentgens).

(5) The length of each generation is in the P and bb populations exactly three weeks, but in R exactly two weeks.

(6) Flies collected for making different kinds of tests are taken out at random prior to being irradiated. The tested flies are, therefore, not in themselves irradiated but their genome has got an accumulated dose which is the sum of all doses given to their ancestry.

(7) An effect of selection pressure is strongly indicated by the differences in the ease with which it has been possible to carry through the routine work and to keep the populations alive. The general picture may be summarized as follows: (i) The populations with a low selection pressure were much more difficult to keep alive than those with a strong selection pressure. Population bb 25, for instance, was in fact very close to extinction at an accumulated dose of 13 kiloroentgens, and, in order to save it, it was necessary to intercalate one generation without any irradiation. (ii) When the accumulated dose had reached a certain level, the viability of all populations seemed to increase with further increase in accumulated dose; however much quicker so in the populations with strong selection pressure than in those with the low pressure. (iii) When the population bb 25, at an

accumulated dose of 13 kiloroentgens, was near to becoming extinct the population bb 25—a was started with flies from population bb 200. The purpose of this was to see if the high viability of bb 200 would, when transformed to population bb 25—a, decline to the low viability of bb 25. Such a decline was, however, not observed.

(8) In spite of these strong indications of an effect of the selection pressure, the different kinds of tests so far performed, have not shown any clearcut differences between the populations. These tests have included egglaying capacity, hatchability of eggs, sterility of males, larval competitions, number and spread into the populations of lethal genes.

(9) Two new populations which, at least for a limited number of generations, will be closely followed and compared are started. One of these, bb 25—c, takes it origin from a non irradiated wild type strain which just has been made homozygous for the chromosomes number 1, 2 and 3, but of another ancestry than that mentioned under point 2 above. The other population, bb 25—d, is derived from bb 200 at an accumulated dose of 29 kiloroentgens and of a very good viability.

(10) When making tests on »survival rates» females and males with the genotype Cy L/+ were mated, either with a common wild type chromosome (homozygous tests) or with wild type chromosomes of different ancestry (heterozygous tests). In the first case undertaken of these tests (at accumulated doses of 7, 8.5, 5, and 5 kiloroentgens respectively for P 25, bb 25, P 200 and bb 200) it was found that the number of flies in the progeny phenotypically Cy L was larger in P 200 and bb 200 (i.e. in the populations with strong selection pressure) than in P 25 and bb 25 (i.e. in populations with low selection pressure). Whatever the explanation may be it seems necessary to conclude marked dominance effects of the irradiated wild type chromosomes. A corresponding result was not found in tests on survival rates performed at later occasions.

(11) Before any valid conclusions may be drawn from these experiments on the effects of differences in selection pressure on irradiated populations, these populations must be prosecuted and carefully studied for several more generations.

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THE EFFECT OF CHRONIC GAMMA RADIATION ON THE HATCHABILITY OF CHICKEN EGGS

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THE aim of the present experiments was to determine the gamma-ray dosage to chicken eggs that would be most suitable for producing changes on chicks hatched from the eggs, and to compare the effects of the same dosage administered over varying periodes. The investigation was carried out at the Agricultural College of Norway during the summer of 1957.

EXPERIMENTAL PROCEDURES

Two experiments were carried out. In either, eggs from the White Leghorn strain of the Institute of Poultry and Fur Animals were used. In the first experiment 180 eggs, laid on the day preceding the irradiation were divided randomly into six equal groups. One group was kept as control and the other five were irradiated with doses ranging from 3 700 to 270 r. The irradiation time for all groups was 6 hours.

In the second experiment 1 136 eggs were used. The eggs were laid during the last 8 days before the irradiation started. In this experiment the eggs were divided into 16 groups with 71 eggs in each. Eggs laid on the same day were divided randomly, but all groups received an equal number of eggs laid on the same day. In this way the average age of the eggs was the same for all groups. One of the 16 groups was kept as control. The other 15 were irradiated with doses of approximately 1 500, 1 000, or 500 r. Each of the 5 groups on the same dosage was given a different irradiation time, namely: (1) 6 hours, (2) 24 hours, (3) 48 hours, (4) 96 hours, and (5) 144 hours.

The irradiation was carried out with gamma-rays from the ^{60}Co source (48 curies) belonging to the Institute of Genetics and Plant Breeding. The isotope was placed in a vertical aluminium tube, and

circular wire baskets for the eggs were arranged at different distances from the source (Fig. 1). The distance of each basket was determined by means of a graph based on several measurements. When the irradiation had been finished and the eggs removed, the intensity of the irradiation was measured by Moxnes chambers placed in the baskets. At short distances the intensity decreases very rapidly with increasing distance, and the measurements proved that we had not succeeded in placing all the baskets at correct distances. As shown in Table 1, the actual doses were therefore in some cases different from those planned.

The ^{60}Co isotope, set up for plant breeding experiments, was placed in the open air, and a roof was made to protect the eggs from rain and sun. The control groups were kept under similar conditions, sheltered from irradiation by a concrete wall, and so far away from the source that no irradiation was measurable. All eggs were placed in the baskets at the same time for each experiment. In the second experiment the isotope was lowered into a chamber of lead and concrete after the irradiation time of each group had been completed, and the eggs of the group were removed and placed together with the control eggs. The time the isotope was removed was added to the irradiation time for the rest of the groups.

When the irradiation of all groups was finished all eggs were placed in the same incubator. The eggs were candled after six days of incubation, and those without a living embryo were removed. 18 days from the start of the incubation the eggs were again candled, and those with dead embryos were removed. The rest of the eggs were transferred to a special incubator for hatching.

RESULTS

It was clear already at the first candling that the development of the embryos had been slowed down by the irradiation. In the first experiment the eggs were candled also 3 days after incubation. In Group 3 which had received 1 500 r, no embryos were visible at this candling. After 6 days, however, the number of visual embryos was 4. The effect of the irradiation on the rate of development was also studied on embryos from eggs opened on the 23rd day of incubation. Several embryos were still alive, but they were up to 6 days delayed in their development. The normal chicks from the irradiated eggs did also hatch later than those from the control eggs.

It emerges from Table 1 and Fig. 2 that the candling of the eggs

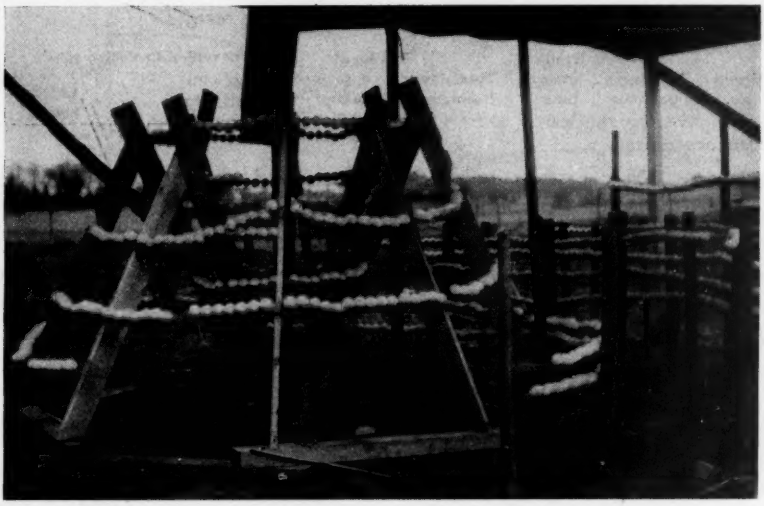

Fig. 1. How the eggs were arranged during the irradiation.

in group A/2 (1 728 r) resulted in only one visible embryo. It is also possible that this single egg had been protected in some way. In the groups receiving 1 500—1 584 r, the number of eggs with visual embryos averaged 8 per cent of that of the control eggs. The 1 200—1 500 r groups had 27 per cent and the 900—1 200 r groups 60 per cent on the average. The number of visual embryos in the groups irradiated with 500—600 r, was 64 per cent of that in the control, and 270 r did not lower the number of visual embryos at all.

As to the hatching results no egg receiving more than 1 080 r hatched. For the 930—1 080 r groups the hatch was on the average 6 per cent of that obtained for the control groups. The results for the 500—600 r groups was on the average 60 per cent of those for the control, and the hatching of the single group of 30 eggs, irradiated with 270 r, was 96 per cent of that obtained for the control.

In previous investigations radiation effects have chiefly been studied after acute radiation treatments over a short period of time. In plant experiments MIKAELSEN (1954) found that the intensity of the irradiation was a more important factor in the yield of aberrations in root tips of *Tradescantia paludosa* than the total dose given. In the present experiments no significant difference concerning the effect of different intensity of irradiation was found when the total dose remained ap-

TABLE 1. *Candling and hatching results.*

Group No.	Roentgens per hour	Irradiation time Hours	Total dose Roentgens	No of. eggs incubated	Per cent of incubated eggs[1] Eggs with embryo development	Chicks hatched
First experiment						
1	620	6	3 700	30	0 (0— 16)	0 (0— 16)
2	420	6	2 500	30	0 (0— 16)	0 (0— 16)
3	250	6	1 500	30	13 (3— 36)	0 (0— 16)
4	155	6	930	30	93 (72—100)	7 (0— 28)
5	45	6	270	30	97 (78—100)	93 (72—100)
K	—	—	—	30	97 (78—100)	97 (78—100)
Second experiment						
A/1	332	6	1 392	71	14 (5— 28)	0 (0— 8)
A/2	72	24	1 728	71	1 (0— 11)	0 (0— 8)
A/3	33	48	1 584	71	0 (0— 8)	0 (0— 8)
A/4	14	96	1 344	71	23 (12— 40)	0 (0— 8)
A/5	10.4	144	1 498	70	14 (5— 28)	0 (0— 8)
B/1	264	6	1 584	71	11 (3— 25)	0 (0— 8)
B/2	45	24	1 080	71	34 (20— 50)	1 (0— 11)
B/3	25	48	1 200	70	51 (35— 68)	0 (0— 8)
B/4	10.4	96	998	71	48 (32— 64)	0 (0— 8)
B/5	6.9	144	994	71	45 (31— 62)	3 (0— 14)
C/1	160	6	960	71	82 (67— 92)	16 (7— 30)
C/2	25	24	600	70	89 (77— 97)	60 (44— 75)
C/3	10.4	48	499	71	87 (74— 96)	45 (31— 62)
C/4	5.21	96	500	71	85 (71— 95)	45 (31— 62)
C/5	3.47	144	500	71	87 (74— 96)	45 (31— 62)
K	—	—	—	69	94 (83— 99)	81 (67— 91)

proximately the same. (Table 1.) This divergence from the results referred to above, may depend on the lower activity in stored eggs compared with the rapid cell division in root tips.

All the chicks that hatched, were by a gross examination classified as normal at hatching time. The eggs that had not been hatched were opened on the 23rd day of incubation, and the embryos were examined. The following 12 out of 130 embryos studied, showed distinct abnormalities:

First experiment

Group No. 3, irradiated with 1 500 r. One embryo was examined. It showed a large, almost spherical cerebral hernia. Upper beak twisted

[1] 99 % confidence interval.

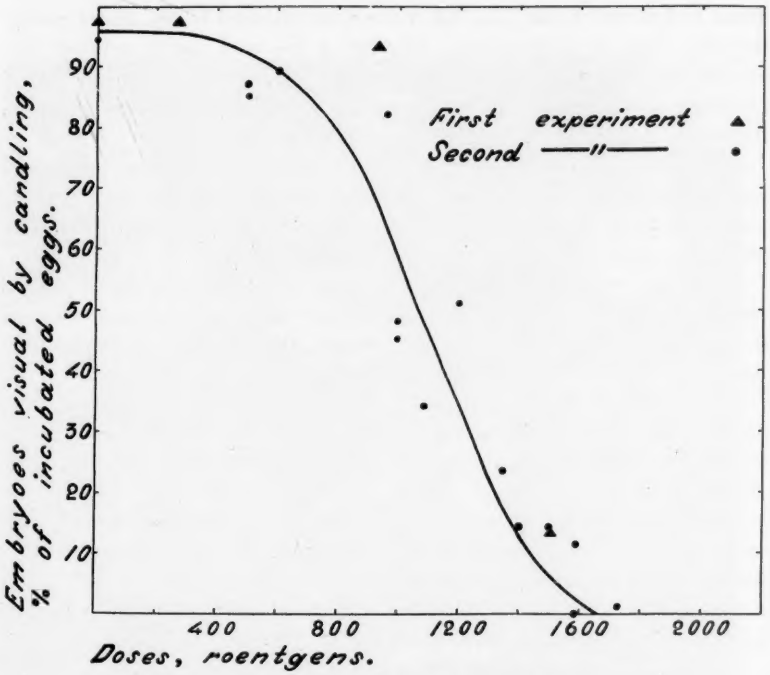

Fig. 2. Effects of various doses on embryo development.

toward the right side. Length of upper beak 7.5 mm. Lower beak was straight, but abnormally long, 12.5 mm. The right eye was missing and the left one extremely underdeveloped. Finally the embryo had umbilical hernia (Fig. 3 A).

Group No. 4, irradiated with 930 r. Fifteen embryos were examined, but only two showed distinct abnormalities. One of the embryos had cerebral hernia, the other was almost completely naked (Fig. 3 B).

Second experiment

Group No. A/4, irradiated with 1 344 r. One embryo was examined. The right foot was extremely abnormal. The size of the tibia and the more distal bones of the limb, except those of the second and the third toe, were about half normal size. The shape was also abnormal. The tibia ended at the distal epiphysis, and had consequently no distal joint surface. The metatarsus was united to the tibia about 5 mm from the

distal end of this bone. The No. 1 toe was attached to the distal end of the tibia (not to metatarsus). The No. 4 toe was much reduced and attached to the second phalange of the No. 3 toe. Only the distal phalange was present. The second and the third toe were closely joined the whole length of the shorter toe by a web (Fig. 3 C).

Group No. B/3, irradiated with 1 200 r. Four embryos were examined and two of these were abnormal. The abdominal cavity of one of them was completely open from the keel bone to the vent (Fig. 3 D). The other had a process at the back of the head (Fig. 3 E).

Group B/4 irradiated with 998 r. Two of the four embryos examined showed distinct abnormalities. One of them had only sparse strands of down (Fig. 3 F). On the left foot of the other the No. 2 toe pointed medially, and not forward as normal (Fig. 3 G).

Group B/5, irradiated with 994 r. Five embryos were examined. The upper beak of one of them was approximately 2 mm shorter than the lower one. This case was very similar to those reported by LANDAUER (1941) (Fig. 3 H).

Group C/1, irradiated with 960 r. Seventeen embryos examined. One of these had a process at the back of the head. Microscopic examination did not show any distinct abnormalities.

Group C/3, irradiated with 499 r. Fourteen embryos were examined and one of these had cerebral hernia (Fig. 3 I).

Group C/4, irradiated with 500 r. One of the fifteen embryos examined had a short upper beak.

SUMMARY

In two experiments a total number of about 1 300 eggs were irradiated with gamma-rays from a ^{60}Co source and exposed to doses varying from 3 700 r to 270 r. The irradiation time ranged from 6 to 144 hours.

Irradiated eggs hatched more slowly than did the control eggs. In eggs irradiated with doses exceeding 1 584 r no embryos were visible by candling after six days of incubation. No eggs irradiated with more than 1 080 r hatched. No significant difference concerning the effect of different intensity of irradiation was found when the total dose remained approximately the same.

Twelve abnormal embryos from eggs that did not hatch have been described.

Acknowledgement. — The author wishes to express his thanks to Prosector N. KOPPANG for his help with the examination of the abnormal embryos.

Fig. 3. Abnormal embryos from eggs opened at the end of incubation.

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L'ACTION DU MYLERAN (DI-MÉTHANE-SULFONYLOXY-BUTANE) SUR LES CHROMOSOMES CHEZ *HORDEUM SATIVUM* ET CHEZ *VICIA FABA*

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I. INTRODUCTION

PARMI les questions qui ont passionné les cyto-généticiens durant les dernières années, la production de mutations dirigées fut certainement l'une des plus attachantes. Obtenir des formes nouvelles à volonté est un rêve que l'homme caresse depuis longtemps. Les mutations sont généralement considérées comme se produisant au hasard et l'utilisation intensive des radiations ionisantes dans la genèse des mutations n'a eu pour effet que de renforcer cette idée. Cependant, l'avènement des mutagènes chimiques devait livrer des espoirs de diriger les mutations. Ce fut l'opinion d'EHRENBERG, GUSTAFSSON et VON WETTSTEIN (1956) que les mutations produites par les toxiques ne sont pas du tout réparties au hasard mais que le spectre de mutations change d'un toxique à l'autre. Les nombreux travaux réalisés dans ce sens par les chercheurs de l'Ecole Suédoise ont fortement étayé cette conception.

D'un autre côté, la notion de spécificité s'est imposée de plus en plus pour diverses substances, dans leur aptitude à casser les chromosomes. Parmi les molécules chimiques efficaces dont le généticien peut actuellement disposer, le Myleran est l'une des dernières qui soit à l'étude. EHRENBERG, GUSTAFSSON et ses collaborateurs rassemblent actuellement les résultats obtenus grâce à ce toxique et ceux-ci sont très prometteurs. Le Myleran se montre apte à produire un pourcentage

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élevé de mutations intéressantes et induit des taux élevés de plantes et d'épis stériles. En présence de ces résultats génétiques, il était donc opportun d'avoir des précisions sur les modalités de cassures chromosomiques causées par cette substance. C'est ce que nous nous proposons au cours de ce travail.

Le 1-4-di-methane-sulfonyloxy-butane [$CH_3 \cdot SO_2O(CH_2)_4O \cdot SO_2 \cdot CH_3$] breveté par Burroughs Welcome and Cy. à Londres sous le nom de Myleran a déjà été utilisé à maintes reprises en hématologie. HADDOW et TIMMIS (1953) ont utilisé plusieurs esters sulfoniques. Le composé possédant deux groupes fonctionnels $O\text{-}SO^2\text{-}CH^2$ et une chaîne de groupes (CH^2) a été essayé comme inhibiteur de croissance du carcinome de Walker 256 du rat. Ces composés mésyloxy- exercent aussi une action dépressive sur les moelles osseuses, d'où leur usage fréquent dans le traitement des leucémies. Dans ce cas, seules les cellules de la série myéloïde sont touchées à l'exclusion des cellules de la série lymphoïde et c'est là un premier exemple d'action élective de cette substance. Ce sont aussi HADDOW et TIMMIS qui ont observé que c'est le composé possédant 4 groupes (CH^2) qui montre une activité maximale. C'est le composé qui nous intéresse dans ce travail.

II. MATÉRIEL ET MÉTHODE

Nous avons utilisé des graines de *Hordeum sativum* de la variété *Bonus* et des graines de *Vicia faba* d'une variété commerciale (Åkerböna, Weibull). Ce sont les mêmes variétés qui nous ont servi dans l'étude de l'action comparée de plusieurs toxiques. Les graines sont utilisées sèches, sans germination préalable et ce, afin, croyons-nous, d'accroître la pénétration du toxique à l'intérieur de la graine.

Le Myleran II (di-méthane-sulfonyloxy-butane) qui provient des laboratoires de la »Chester Beatty Research Institute» (par l'intermédiaire de L. EHRENBERG) a été utilisé aux doses de 20 mgr., 40 mgr. et 80 mgr. pour 100 cc. d'eau distillée. Les solutions sont préparées extemporanément avant leur emploi. La dissolution de la substance est très malaisée; afin de la faciliter et de l'accentuer, nous avons porté la solution à la température de 45° C. Pendant la durée de la dissolution, jamais la température n'a dépassé 45°. Il est nécessaire d'agiter fréquemment la solution de Myleran pendant la dissolution et pendant la durée du traitement.

Les temps de traitement sont de 45 min., 1 1/2 h., 3 h., 6 h., 12 h. et 24 h. Pour *Vicia faba*, le temps de 24 h. n'a pas été retenu en raison de

la très forte mortalité des graines et aussi du fait que l'inhibition mitotique presque totale rend l'étude cytologique fort malaisée. Il est cependant possible que des graines puissent survivre après ce temps de traitement et présentent un certain intérêt du point de vue génétique. Après traitement, les graines sont soigneusement lavées à l'eau courante puis séchées et ensemencées sur papier filtre en boîtes de Petri. Celles-ci sont placées à la température de 20-22° C.

Nos investigations portent sur les pointes des racines principales chez *Vicia* et sur les pointes des 5 premières racines chez *Hordeum*. Nous avons pu généralement identifier les trois premières mitoses consécutives au traitement. Ultérieurement, il est impossible d'avoir une idée des rythmes mitotiques avec quelque certitude étant donné la possibilité de désynchronisation des mitoses, celle-ci différant dans chaque cas. En vue d'étudier le mode de disparition des lésions pendant un temps suffisamment long, des temps arbitraires ont dû être choisis. Par raison pratique, nous les avons choisis identiques chez *Hordeum* et chez *Vicia*, sachant cependant qu'ils ne correspondent probablement pas au même stade de développement.

Les fixations des pointes radiculaires sont réalisées à l'alcool acétique (3-1) pendant 24 h. au plus et colorées par la méthode de Feulgen.

III. RÉSULTATS

A. Observations des effets primaires chez Hordeum et chez Vicia

Les effets dits primaires ou physiologiques de la substance se manifestent principalement au cours de la première mitose après le traitement. Cependant, il n'est pas rare de constater une persistance de ces effets au cours des mitoses successives. Leur expression est généralement fort inconstante. En règle générale, les chromosomes traités sont fortement colorés excepté en certaines zones qui, chez *Vicia*, peuvent être identifiées comme étant hétérochromatiques.

Les lésions se révélant lors de la première mitose consistent tout d'abord

1°) en malformations nucléaires; on observe que les contours des noyaux sont fréquemment ondulés à tel point que, parfois, ils simulent l'apparition de véritables micronoyaux appendus aux noyaux qui ont subi le traitement.

2°) de plus, on constate la présence d'une certaine érosion chromosomique particulièrement en anaphase. Cette érosion est néanmoins très

localisée. Elle est beaucoup plus discrète chez *Vicia* que chez *Hordeum* mais toutefois, on peut l'observer à toutes les doses (20-80 mgr.) et pour tous les temps d'action du toxique.

3°) en collage (stickiness) des chromosomes en méta- et en anaphase. La variabilité du phénomène est grande non seulement d'un traitement à l'autre (pour 4 traitements dans les cas des 2 espèces) mais encore dans les différentes racines étudiées. Elle peut être présente, dans certains cas, jusque dans 30 % des métaphases et des anaphases. Dans la majeure partie des cas, elle est restreinte à certains groupes de cellules. Cette observation est identique chez *Hordeum* et chez *Vicia.*

En métaphase (la première après le traitement), nous avons noté que les chromosomes fissurés restent collés; seuls, les bouts terminaux sont séparés. Dans d'autres figures métaphasiques, les chromatides sont écartées l'une de l'autre mais restent collées en des régions très proches des centromères; ces régions sont fortement colorées par la réaction de Feulgen. Les zones centromériques sont, par contre, intimement collées.

Après séparation des chromatides, les régions paracentromériques dont nous venons de parler restent très fréquemment réunies par de très fins filaments colorés. Chez *Hordeum*, la persistance de ces petits filaments est rare, mais chez *Vicia*, on observe fréquemment leur présence particulièrement dans le chromosome M (métacentrique). Dans celui-ci, ce sont les deux chromomères juxta-centromériques qui sont effilés. Ces filaments peuvent joindre les régions juxta-centromériques des deux chromatides mais, plus fréquemment, deux filaments partant des régions juxta-centromériques sont orientés dans le sens des fibres fusoriales. Lors de la première mitose après le traitement, les »pseudo-ponts» sont pratiquement inexistants chez *Hordeum*. On en voit par contre, chez *Vicia faba*, avec une fréquence d'environ 0,12 pour cent anaphases.

Chez *Vicia*, dans plusieurs anaphases (pour tous les traitements), on a pu continuer à observer les filaments ténus unissant les zones centromériques ou bien d'autres zones chromosomiques dans ce cas, toujours identifiées à des zones hétérochromatiques. Nous distinguons bien ces filaments des »pseudo-ponts» dont nous venons de signaler l'existence et, évidemment, des ponts vrais dont l'aspect est totalement différent. La fréquence de ces petits filaments unissant les pôles anaphasiques n'excède guère 0,5 % des anaphases.

Notons en plus, qu'en anaphase, la constriction nucléolaire de *Vicia* montre souvent un aspect particulier. Beaucoup plus longue que d'habitude, elle se présente sous la forme d'un filament légèrement chroma-

tique fort semblable aux filaments décrits plus haut. Sur la longueur de ce filament, on observe quelquefois 1 à 3 corpuscules légèrement colorés.

Nous avons déjà insisté sur le fait que ces phénomènes décrits sous le nom d'effets primaires peuvent être rémanants au cours de plusieurs mitoses. Remarquons également qu'au cours de la première mitose, on peut compter un certain nombre de fragments chromosomiques, tant chez *Hordeum* que chez *Vicia*. Certains de ces fragments étant attachés, peuvent évidemment être la conséquence d'un »collage» et satisfaire à la définition de fragments primaires. Cependant, puisque des effets primaires résiduels peuvent être observés au cours des mitoses successives, il est bien difficile de définir l'origine précise de chaque fragment. Aussi, avons-nous traité la question des cassures produites par le toxique indépendamment des effets primaires, au paragraphe suivant.

B. Quelques données cytologiques quantitatives concernant l'action du Myleran chez Hordeum sativum

Comme nous l'avons mentionné dans le paragraphe réservé à l'exposé de la méthode, les taux de cassures ont pu être évalués pendant les trois premières mitoses consécutives au traitement. Après une période de 200 heures, nous n'avons pu observer aucun fragment. Il existe cependant alors des chromosomes plus courts et cette éventualité sera envisagée ultérieurement.

Les tableaux I et II montrent les modifications quantitatives des cassures méta- et anaphasiques durant les trois premières mitoses chez *Hordeum sativum*.

Le tableau I montre que:

1) le taux de cassures par cellule augmente quand le temps de traitement augmente de 45 minutes à 12 heures; au-dessus de ce temps d'action, les mitoses sont dégénératives;
2) le maximum de cassures est observé entre 60 et 90 heures après le traitement, soit lors de la seconde mitose consécutive au traitement. Après ce moment, le taux de cassures diminue.

Pour le tableau II, les conclusions tirées du tableau I restent valables.

De l'examen des cassures consécutives à l'action du Myleran chez *Hordeum*, on peut déduire que:

1°) dans les limites des doses utilisées (20 mgr. à 80 mgr.), le taux de cassures est constant. (Le chiffre donné représente la moyenne statistique de 4 expériences);

TABLEAU I. *Modifications des taux de cassures métaphasiques chez Hordeum sativum.*

Nombre de cassures par métaphase (sur 500 métaphases environ)

Mitoses	Durée du traitement (h.)						
	0	3/4	1 1/2	3	6	12	24
X_1 (30—40 h.)	—	0.025	0.037	0.046	0.060	0.081	0.10 (les. prim.)
X_2 (60—90 h.)	—	—	0.076	0.391	0.539	0.800	dégénératif
X_3 (100—150 h.)	—	—	0.011	0.046	0.274	0.528	mort

TABLEAU II. *Modifications des taux de cassures anaphasiques chez Hordeum sativum.*

Nombre de cassures par anaphase (sur 500 anaphases environ)

Mitoses	Durée du traitement (h.)						
	0	3/4	1 1/2	3	6	12	24
X_1 (30—40 h.)	—	0.02	0.06	0.085	0.09	0.10	0.21
X_2 (60—90 h.)	—	—	0.01	0.143	0.467	0.545	dégénératif
X_3 (100—150 h.)	—	—	—	0.03	0.036	0.012	mort

2°) le taux de cassures augmente avec le temps d'action du toxique, la dose léthale étant atteinte pour 24 heures d'action;

3°) le maximum de cassures est observé lors de la seconde mitose après le traitement et, par conséquent, la substance diffuse lentement dans les milieux intracellulaires jusqu'à son point d'impact;

4°) après la seconde mitose, les fragments sont éliminés.

Nous avons ainsi observé que, 200 heures après le traitement, aucun fragment n'est visible.

En examinant les racines des plantes traitées pendant des temps d'action croissants, nous avons noté qu'il existe une inhibition mitotique marquée. Nous avons alors estimé les taux du taux. Ceux-ci sont exprimés, dans le tableau III en pourcentage de mitoses du témoin.

Le tableau III indique que la substance inhibe les mitoses d'autant plus fortement que le temps d'action du toxique est plus élevé. Cependant, cette inhibition est surtout marquée pendant les 40 premières

TABLEAU III. *Modifications des taux de mitoses chez Hordeum sativum (en % du témoin).*

(sur 500 cellules environ)

Mitoses	Durée du traitement (h.)						
	0	3/4	1 1/2	3	6	12	24
X_1 (30—40 h.)	100	76.2	75.4	55.4	31.5	23.0	20.0
X_2 (60—90 h.)	100	92.3	81.4	71.8	56.4	44.1	5.1 dégénératif
X_3 (100—150 h.)	100	92.6	83.1	75.0	56.7	52.7	mort

heures après le traitement. Ultérieurement, il y a un relèvement de l'activité mitotique d'autant plus marqué que le taux était bas au début et, par conséquent, que le temps d'action du toxique était élevé, sauf pour la dose léthale de 24 heures.

A partir de la seconde mitose après le traitement, nous avons aussi pu observer des micronoyaux formés à partir de fragments chromosomiques isolés dans le cytoplasme lors de l'anaphase de la première division. Le tableau IV résume les observations effectuées à ce sujet.

Le tableau IV montre que:

1) les micronoyaux apparaissent en seconde mitose (toutes les phases mitotiques sont rassemblées);
2) le taux de micronoyaux est maximum pendant la période de 60 à 90 heures. Après ce temps, les taux diminuent;
3) les taux de micronoyaux sont proportionnels aux temps d'action du toxique.

TABLEAU IV. *Modifications des taux de micronoyaux chez Hordeum sativum.*

(% sur 400 cellules environ)

Mitoses	Durée du traitement (h.)						
	0	3/4	1 1/2	3	6	12	24
X_1 (30—40 h.)	—	—	—	—	—	—	—
X_2 (60—90 h.)	—	—	0.23	0.68	1.10	2.2	dégénératif
X_3 (100—150 h.)	—	—	—	0.15	0.92	1.3	mort

Comme pour les fragments, nous avons observé qu'après 200 heures, plus aucun micronoyau n'est repérable.

Devant cet état de choses, nous avons posé la question suivante: Le Myleran est-il capable d'inhiber la formation de toutes les réunions ou seulement de certains types de réunions, celles-là mêmes qui conduisent à la formation de ponts? L'analyse de cette question a été réalisée chez *Vicia faba*, matériel plus commode pour ce genre de travail. Néanmoins, chez *Hordeum*, un certain nombre de cassures chromatidiques [cassures B′ de DARLINGTON et UPCOTT (1941)] a été constamment observé. Etant donné le rapport qui peut exister entre ces cassures et les réunions, un relevé en a été effectué. Il est résumé par le graphique I (p. 425) qui est commenté p. 424.

C. Analyse des résultats précédents

Un fait qui attire l'attention dans l'action du Myleran sur les chromosomes de *Hordeum*, est son aptitude accusée à casser les chromosomes si l'on compare ces données à celles qui ont été relevées pour différents toxiques appartenant à diverses classes chimiques de même que pour les radiations ionisantes. Mais un autre fait beaucoup plus important, est la carence complète de ponts chromosomiques. Durant tous les cycles mitotiques au cours desquels les taux de cassures ont été suivis, aucun pont vrai n'a jamais été rencontré chez *Hordeum*, pour un total de 4 expériences. Quant aux »pseudo-ponts» dont nous avons antérieurement mentionné l'existence en même temps que les effets physiologiques du toxique, rappelons qu'ils sont fort rares.

Dans le tableau V, nous avons rassemblé quelques données concernant l'action de différents toxiques et l'action des radiations ionisantes afin de pouvoir comparer pour divers agents mutagènes, leur aptitude à casser les chromosomes et la possibilité pour ceux-ci, de se réunir; les doses des différents agents ont été choisies arbitrairement (nous avons, autant que possible, figuré celles qui donnent à peu près le même taux de cassures chromosomiques par anaphase).

Il est évident que les rapports $\frac{\text{n. de fragments}}{\text{n. de ponts}}$ n'ont aucune valeur absolue et ne peuvent être retenus qu'à titre de comparaison.

Le tableau V montre que l'on peut distinguer trois classes de toxiques parmi ceux utilisés eu égard à leur action sur les chromosomes:

a) les toxiques qui donnent des cassures chromosomiques et des ponts; ils forment la majorité des substances employées;

TABLEAU V. *Comparaison entre les taux de fragments et de ponts anaphasiques (Mitoses X_2) par cellule pour différents toxiques et pour les radiations ionisantes (chez Hordeum sativum).*

	Nébularine 300 mgr. % 4 h.	Myleran 80 mgr. % 3 h.	8-éthoxy-caféine 150 mgr. % 6 h.	Ethylène-oxyde 200 mgr. % 24 h.	Diepoxy-butane 113 mgr. % 2 h.	Ethylène-imine 40 mgr. % 2 h.	Rayons X 4 Kr.	Neutrons 400 rep.
Nombre de fragments par anaphase	0	0.190	0.080	0.151	0.06	0.461	0.186	0.20
Nombre de ponts par anaphase	0	0	0.080	0.14	0.05	0.246	0.271	0.155
Nombre d'anaphases examinées	1.000	500	200	200	200	200	250	200
Rapport fragments/ ponts	—	∞	1	1.08	1.2	1.87	0.69	1.29

b) le Myleran qui est, jusqu'à présent, la seule substance donnant des cassures et pas de ponts vrais;

c) la nébularine qui ne donne ni cassure, ni pont. Cette substance avait été retenue en raison du fait qu'elle induit un certain nombre de mutations visibles (EHRENBERG, GUSTAFSSON et VON WETTSTEIN, 1956). D'autres substances du même type sont actuellement à l'étude.

Devant cet état de choses, nous nous sommes demandé si, chez *Hordeum*, le Myleran était capable de produire uniquement des cassures sans permettre les réunions ou si cette assertion pouvait être soumise à quelques restrictions. La difficulté inhérente au matériel n'a pas permis une analyse cytologique détaillée de cette question mais toutefois nous pouvons noter que si des réunions existent, elles sont rares.

Nous devons néanmoins accorder une attention particulière à un problème que nous n'avons pas soulevé jusqu'à présent. Quand, dans les tableaux I et II, nous parlons de fragments chromosomiques méta- et anaphasiques, nous englobons sous ce concept, les cassures dites chromosomiques (B″) et chromatidiques (B′) sans préciser les pourcentages relatifs des deux types de cassures. Lorsque nous analysons les possibilités de réunions, il est dès lors indispensable de voir si le Myleran est capable de provoquer un pourcentage appréciable de cassures chromatidiques (B′).

Le taux de cassures chromatidiques a été relevé pendant la période

où le toxique montre un maximum d'action (75 h. après le traitement). Le graphique I montre que le taux de cassures chromatidiques augmente avec le temps d'action de la substance. Il montre aussi que la proportion de cassures chromatidiques augmente par rapport aux cassures isochromatidiques (chromosomiques). Nous avons en plus observé qu'un pourcentage élevé de ces cassures B′ était localisé dans la partie terminale des bras chromosomiques. Ce pourcentage est figuré sur le graphique I en hachuré. En présence d'une localisation préférentielle des cassures chromatidiques, une localisation de tous les types de cassures devait être tentée.

Nous avons pu localiser les cassures produites par le Myleran dans le génome de *Hordeum* bien que la morphologie des chromosomes désignés sous le nom de II, III et IV, soit fortement semblable et entache, dès lors, la répartition des cassures, d'une certaine erreur.

L'examen du graphique II montre que:

1) la répartition des cassures est loin de se faire au hasard. Pour les chromosomes VI et VII, on peut comparer avec l'action des radiations ionisantes (J. et M. MOUTSCHEN-DAHMEN, 1958);
2) on note une grande fréquence de localisations,
   a) dans les régions juxta-centromériques (figurées en noir),
   b) dans les régions proches des constrictions satellitiques (figurées en hachuré) et des constrictions tertiaires (figurées en pointillé).

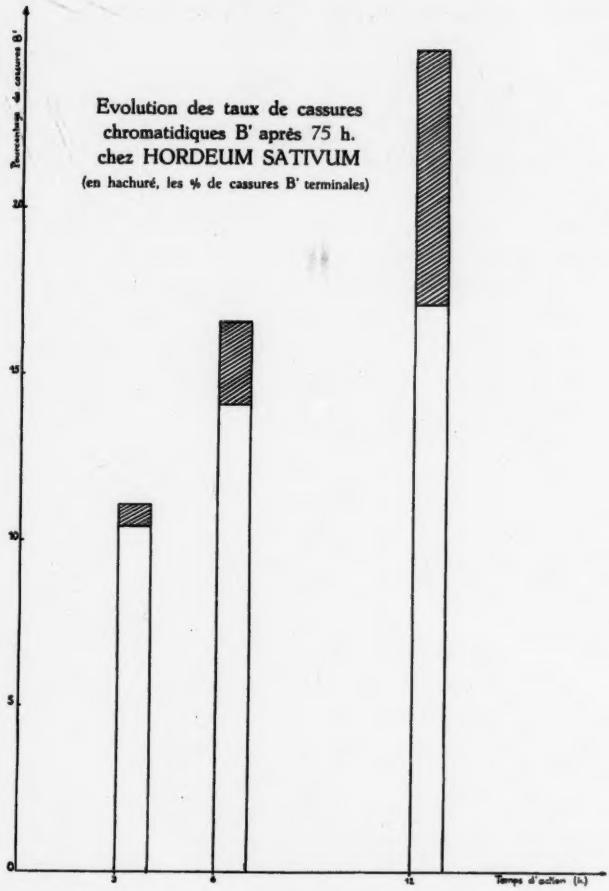
La fréquence des cassures figurées en blanc sur le graphique se rapporte à un bras chromosomique entier à l'exclusion des zones précédentes et sans qu'il soit possible de préciser les localisations en des lieux de ces bras chromosomiques.

Les observations cytologiques nous ont permis de montrer que les cassures localisées se trouvent en des zones très voisines des constrictions et non pas dans les constrictions elles-mêmes. Les lésions produites dans les zones juxta-centromériques se révèlent très tôt (35 h. après le traitement). Nous les avons décrites avec les effets primaires. Parmi les différents toxiques utilisés chez *Hordeum*, seule l'éthylène-imine nous a donné des lésions assez comparables.

En résumé, chez *Hordeum sativum*,

1°) les doses de Myleran comprises entre 20 et 80 mgr. donnent les mêmes taux de lésions;

2°) les taux de cassures aussi bien anaphasiques que métaphasiques augmentent quand le temps d'action du toxique augmente (de 45 minutes à 12 heures d'action);

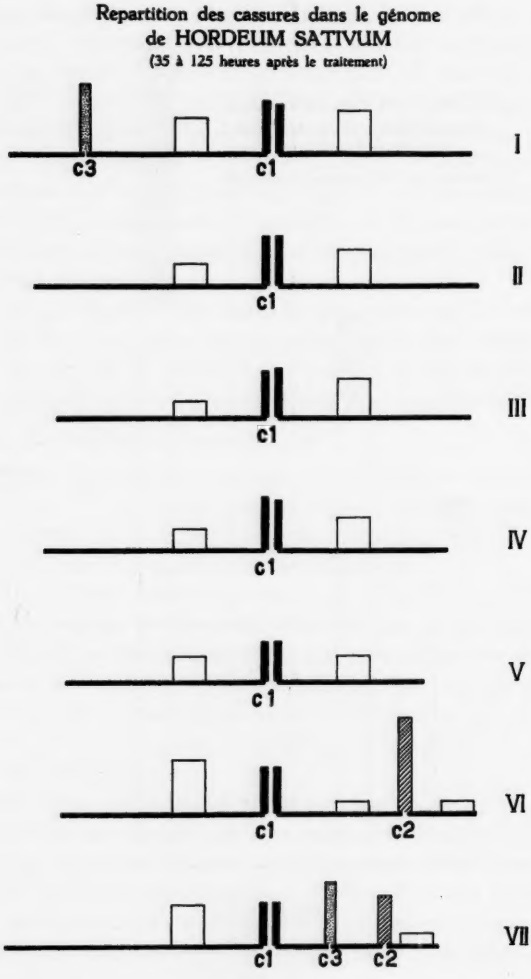


Graphique I.

3°) le taux maximum de cassures est obtenu lors de la seconde mitose consécutive au traitement; ensuite, il diminue; après 200 heures, on n'observe plus de cassures;

4°) malgré le nombre élevé de cassures, aucun pont vrai n'a été observé pendant les premières mitoses consécutives au traitement;

5°) la répartition des cassures dues au Myleran est nettement systématisée.



Graphique II.

Les cassures ont été localisées avec une plus grande fréquence

a) dans les régions juxta-centromériques,

b) dans les régions adjacentes aux constrictions satellitiques et aux constrictions tertiaires.

D. Quelques données cytologiques quantitatives concernant l'action du Myleran chez Vicia faba

Les modifications des taux de cassures produites chez *Vicia* ont été suivies pendant un temps plus long que chez *Hordeum* en raison du fait qu'elles se maintiennent plus longuement. Les tableaux VI et VII résument les modifications des taux de cassures pendant une période de développement de 350 heures après le traitement.

TABLEAU VI. *Modifications des taux de cassures métaphasiques chez Vicia faba.*

Nombre de cassures par métaphase (sur 500 métaphases environ)

Temps après le traitement (h.)	Durée du traitement (h.)					
	0	3/4	1 1/2	3	6	12
35	—	0.43	0.21	0.19	—	—
75	—	0.63	0.5	0.47	0.59	1.92
125	—	0.60	0.24	0.27	0.28	—
200	—	0.02	0.16	0.21	0.48	—
250	—	0.04	0.15	0.18	0.32	—
350	—	—	—	0.08	0.17	—

TABLEAU VII. *Modifications des taux de cassures anaphasiques chez Vicia faba.*

Nombre de cassures par anaphase (sur 500 anaphases environ)

Temps après le traitement (h.)	Durée du traitement (h)					
	0	3/4	1 1/2	3	6	12
35	—	0.09	0.01	0.01	—	—
75	—	0.36	0.31	0.34	0.40	0.72
125	—	0.08	0.13	0.19	0.30	—
200	—	0.01	0.04	0.12	0.20	—
250	—	—	0.03	0.08	0.14	—
350	—	—	—	0.03	0.04	—

Le tableau VI montre que:

1) il n'y a pas, comme chez *Hordeum*, de proportionnalité entre les taux de cassures et les temps de traitement. On peut dire que, jusqu'à 75 heures inclusivement, cette proportionnalité n'existe pas, c'est-à-dire que, lors des deux premières mitoses consécutives au traitement, les taux de cassures sont du même ordre de grandeur quel que soit le temps d'action du toxique. Rappelons aussi que chez *Vicia faba*, 4 expériences ont été réalisées à des doses allant de 20 mgr. à 80 mgr. p. 100 cc. Ces 4 expériences donnent pratiquement les mêmes résultats.

2) après la seconde mitose consécutive au traitement, une certaine proportionnalité existe entre les taux des cassures et les temps d'action du toxique.

3) pour un temps de traitement de 12 heures, quelques mitoses aberrantes sont observées 75 h. après le traitement mais à ce temps d'action du toxique, les racines dégénèrent. Nous avons aussi remarqué que six cents heures après l'action du toxique, le nombre de cassures observées est inférieur à 0,01 % par métaphase. Nous pensons donc avoir pu suivre le cycle de production de la majorité des cassures, celui-ci s'étendant sur la période de 600 heures.

Le tableau VII montre que les fragments anaphasiques se modifient comme les taux de fragments métaphasiques et les commentaires du tableau VI sont tout à fait applicables au tableau VII.

Cytologiquement, nous avons observé que les fragments anaphasiques sont très généralement de grandes tailles. Comme pour beaucoup de drogues que nous avons utilisées chez *Vicia*, nous avons noté un nombre très réduit de micro-fragments.

Dans le tableau VIII, nous donnons les pourcentages de mitoses exprimés en pourcentage du témoin.

Ce tableau montre que, chez *Vicia*,

1°) au fur et à mesure que la dose augmente, il y a une inhibition progressive des mitoses;

2°) 75 heures après le traitement, le taux de mitoses augmente d'une manière d'autant plus accusée qu'il y avait inhibition;

3°) après la période précédente, le taux de mitoses diminue légèrement et montre une certaine tendance à se stabiliser. Cependant, pour 12 heures de traitement, les organes sont généralement dégénératifs. Le tableau IX indique les modifications des taux de micronoyaux.

Ce tableau IX montre que:

1) lors de la première mitose après traitement, il n'y a pas de micro-

TABLEAU VIII. *Modifications des taux de mitoses chez Vicia faba.*

(en % du témoin)

Temps après le traitement (h.)	Durée du traitement (h.)					
	0	3/4	1 1/2	3	6	12
35	100	90.7	86.4	78.5	35.7	21.4
75	100	99.1	93.8	88.2	59.3	34.4
125	100	98.6	91.3	66	48.6	15.3
200	100	98	89.3	82.7	67.3	0
250	100	95.6	88.6	77.9	65.3	0
350	100	90.1	88.2	68.7	57.2	0

noyaux. Cependant, il n'est pas exclu qu'à ce moment, on observe des fragmentations nucléaires. Les déformations nucléaires sont, en effet, très fréquentes.

2) le taux de micronoyaux est maximal lors de la seconde mitose. La fréquence donnée est, en effet, la moyenne des fréquences observées depuis la prophase jusqu'à la télophase de la seconde mitose après traitement. Il est possible qu'il comprenne en outre des micronoyaux télophasiques de la première mitose ainsi que des noyaux fragmentés.

3) il y a un accroissement du nombre de micronoyaux avec le temps

TABLEAU IX. *Modifications des taux de micronoyaux chez Vicia faba.*

(sur 400 cellules environ)

Temps après le traitement (h.)	Durée du traitement (h.)					
	0	3/4	1 1/2	3	6	12
35	0	0	0	0	0	0
75	0	3.6	5.8	11.7	19.9	0
125	0	1.8	3.1	6.4	14.0	0
200	0	0.85	1.25	3.3	7.0	0
250	0	0.42	0.68	1.1	3.7	0
350	0	0.1	0.1	0.8	0.9	0

d'action du toxique. Le taux de micronoyaux est directement proportionnel au temps d'action, doublé quand le temps d'action est doublé.

4) les taux de micronoyaux décroissent au cours des mitoses successives. On peut croire que le taux est réduit de moitié après chaque mitose ou, tout au moins, après un nombre constant et réduit de mitoses.

E. **Analyse des résultats précédents**

De la même manière que chez *Hordeum*, nous n'avons pas constaté la présence de ponts chez *Vicia*; à l'exception de quelques »pseudo-ponts», aucun pontage interchromosomique n'existe. Etant donné cet état de choses, il était nécessaire de voir si cette substance inhibe réellement toutes les réunions ou seulement certains types. Il fallait donc procéder à l'analyse de différents types de réunions éventuellement détectés.

Nous avons, en fait, pu observer:

1) des réunions entre chromatides-soeurs
2) des anneaux acentriques
3) des cassures chromatidiques.

Le tableau X résume les résultats de l'analyse des types de réunions 1 et 2.

TABLEAU X. *Modifications des taux de réunions chez Vicia faba.*

(20 mgr.-80 mgr.) (45' à 6 h.) par métaphase (sur 500 métaphases environ)

Temps après le traitement (h.)	Réunions entre chromatides-soeurs (acentriques)	Réunions entre chromatides-soeurs (centriques)	Anneaux acentriques	Anneaux centriques	Total	Rapports fragm/réun.
35	0.095	0	0	0	0.095	8.73
75	0.15	0	0.03	0	0.18	12.16
125	0.13	0	0.05	0.01	0.19	7.3
200	0.03	0	0.01	0	0.4	21.7
250	0.01	0	0.02	0	0.03	23.03
350	0.01	0	0.01	0.005	0.025	10.04

Le tableau X nous montre que:

1) aucune réunion entre les chromatides-soeurs des fragments centriques n'a été observée durant la période de 350 heures suivant le traitement;

2) dans le même ordre d'idée, aucun anneau dicentrique n'a été observé. Le taux d'anneaux centriques est extrêmement bas et la possibilité de confusion entre une zone centrométrique et une zone hétérochromatique reste évidemment possible;

3) le taux de réunions des bouts acentriques des chromatides-soeurs (SRC_0 de DARLINGTON) est maximal 75 heures après le début de la germination;

4) le taux de réunions acentriques (SRC_0) augmente (comme en 3) de la première à la seconde mitose puis diminue au cours des mitoses successives s'étendant sur une période de 350 heures.

Du point de vue des cassures chromatidiques, le tableau XI montre que:

1) comparativement au taux de cassures iso-chromatidiques (B″), le taux est assez élevé;

2) ce taux de cassures chromatidiques est du même ordre de grandeur;

a) pour différentes concentrations du toxique;

b) pour différents temps d'action du toxique;

c) pendant une période de 350 heures consécutive au traitement.

Contrairement à ce que nous avons relevé chez *Hordeum*, il n'y a aucune localisation préférencielle de ces cassures B′. Comme chez *Hordeum*, il est indispensable de localiser les cassures dans les diverses

TABLEAU XI. *Modifications des pourcentages de cassures B′ chez Vicia faba.*

Temps après le traitement (h.)	Durée du traitement (h.)				
	3/4	1 1/2	3	6	12
35	8.3	9.2	8.7	8.4	—
75	8.6	9.4	10	10.2	—
125	8.7	8.8	10	9	—
200	8	8.1	8.6	8.4	—
250	9	8.5	7.8	9.2	—

régions du caryotype de *Vicia faba*. Nous avons pu constater que la répartition des cassures est pratiquement la même pour toutes les doses utilisées mais varie au cours du développement des racines. Elle reste la même jusqu'à 125 h. après l'action du toxique mais, après ce temps de développement radiculaire, elle nous a paru fortement modifiée. Les graphiques III et IV (pp. 433—434) résument ces observations. Le graphique III résume les répartitions des cassures de 35 à 125 h. inclusivement et le graphique IV de 200 à 350 heures. Les deux graphiques couvrent donc la période pendant laquelle la majorité des cassures a été observée.

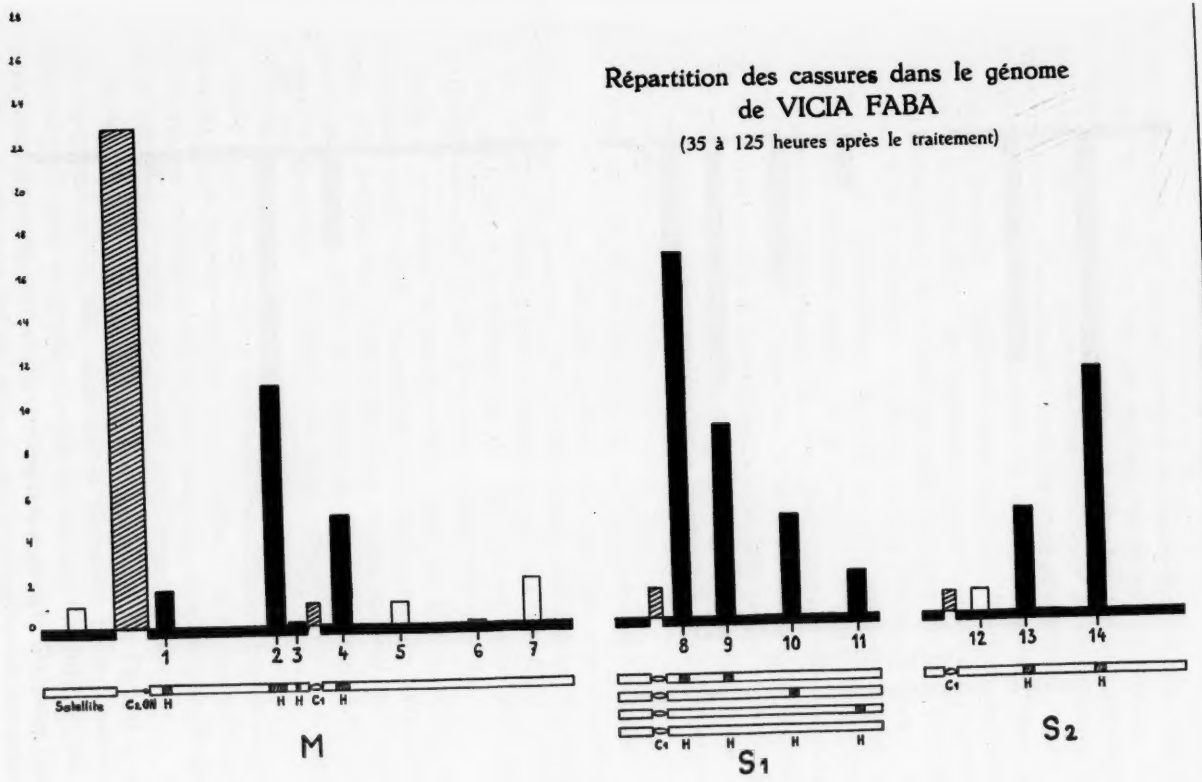
Le graphique III montre qu'il y a une localisation presqu'élective des cassures dans les régions hétérochromatiques du génome. (Localisations figurées en noir sur le graphique.) Les localisations figurées en blanc ne signifient pas nécessairement qu'il s'agisse de segments euchromatiques. Il peut, en effet, s'agir de petites zones hétérochromatiques difficilement repérables. Les localisations figurées en hachuré dans les zones des constrictions sont, en réalité, localisées sur les côtés de ces zones, ce qui montre un certain rapprochement avec *Hordeum*. Il en est particulièrement ainsi de la constriction nucléolaire où les observations répétées nous ont permis de confirmer cette assèrtion.

Du point de vue de la répartition de l'hétérochromatine chez *Vicia faba*, on sait qu'elle a été réalisée par HEITZ, en 1932. DARLINGTON et MC LEISH (1951) et MC LEISH (1953) utilisant la technique de traitement prolongé par le froid des chromosomes de *Vicia* (technique de LA COUR, 1951), a pu distinguer huit zones hétérochromatiques certaines pour un total de 14 segments constituant le contingent haploïde.

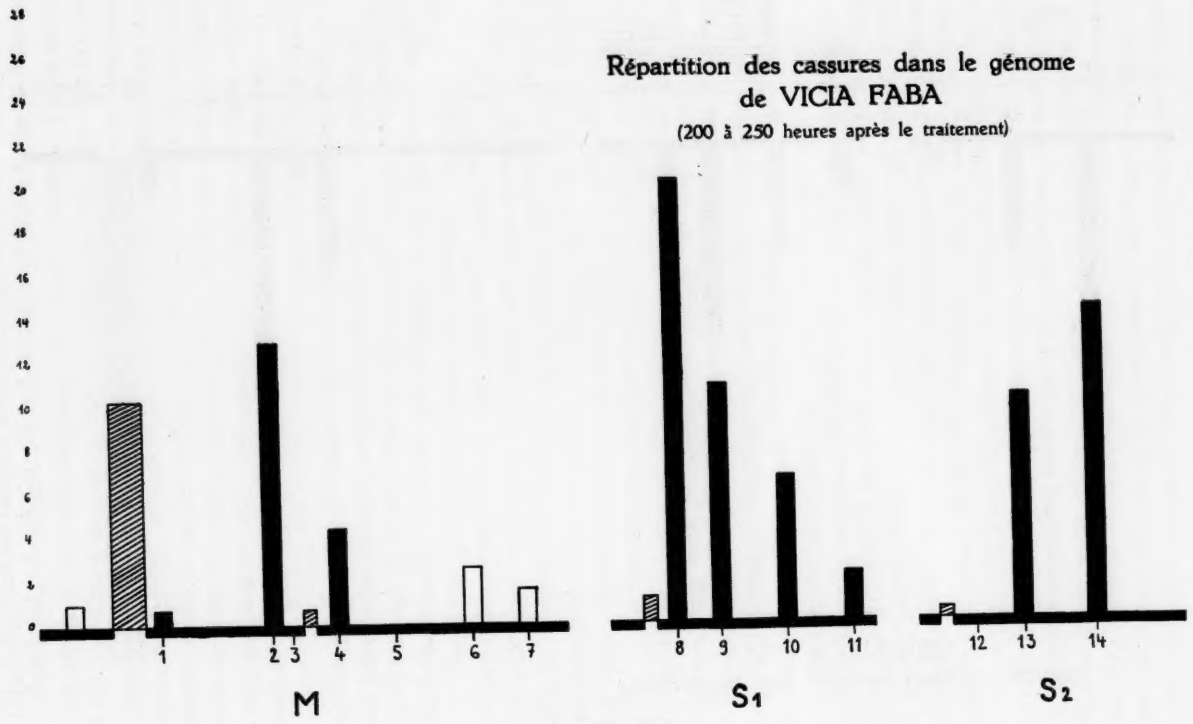
Après traitement par le Myleran, les segments hétérochromatiques sont généralement bien identifiables de sorte que nous n'avons pas cru devoir utiliser la technique de traitement par le froid. Nous avons repris dans cet article, la désignation de MC LEISH au sujet de 14 segments du caryotype de *Vicia faba*.

Le graphique IV montre que la répartition des cassures est également élective après 200 heures (nous n'avons pas inclu les cassures relevées après 350 heures en raison de leur nombre restreint). Dans ce graphique, comme dans le précédent, les fréquences de cassures localisées dans l'hétérochromatine sont extrêmement élevées. Ainsi pour 10 chromosomes (S1+S2), aucune cassure localisée dans l'euchromatine n'a pu être relevée. Pour les chromosomes M, si l'on admet que les segments 6 et 7 puissent contenir de l'hétérochromatine, la fréquence des cassures localisées dans l'euchromatine est réellement fort basse.

Il existe une importante différence par rapport au graphique II. C'est



Graphique III.



Graphique IV.

la forte diminution du taux de cassures localisées dans la constriction nucléolaire. De même, le taux de cassures localisées aux niveaux des constrictions centromériques a tendance à décroître. On sait que toute cassure se produisant au niveau du bras nucléolaire du chromosome M est susceptible de donner naissance à un micronoyau nucléolé.

Il est donc intéressant d'examiner les modifications des taux de micronoyaux nucléolés comparativement aux modifications des taux de cassures du bras distal (des segments 1 à 3 et peut-être du centromère) du chromosome M. Disons que pour les doses de 20 mgr. à 80 mgr. agissant de 45 minutes à 6 heures, nous n'avons pas observé de modifications des pourcentages de micronoyaux nucléolés depuis 75 heures jusqu'à 350 heures après le traitement. Il est de 13,3 % ± 1,6 % de la totalité des micronoyaux. Mais on peut observer que, comparativement à la fréquence de cassures du bras nucléolaire du chromosome M, le pourcentage de micronoyaux nucléolés parait assez faible, ce qui pourrait signifier qu'un pourcentage élevé de satellites isolés ne contient pas l'organisateur nucléolaire. L'observation semble bien confirmer ces vues.

En effet, on peut diviser les types de satellites isolés en 4 classes:

Classe A. — Groupe tous les satellites isolés de dimension normale ou inférieure qui ne possèdent pas d'organisateur nucléolaire. Fréquemment, la zone de l'organisateur nucléolaire a pu être détectée au niveau du bras centrique. Les cassures ont dû, dans ce cas, selon toute vraisemblance, s'effectuer en des lieux très proches des satellites.

Classe B. — Groupe les satellites isolés avec réunions entre chromatides-soeurs acentriques. Ces réunions s'effectuent par les bouts proximaux des chromatides satellitiques. Dans ce cas, comme dans le précédent, les cassures ont été réalisées en des lieux très proches du satellite et l'organisateur nucléolaire est resté accroché avec le fragment centrique.

Classe C. — Le satellite est isolé avec l'organisateur nucléolaire. Celui-ci est séparé du satellite par toute la distance de la constriction secondaire. Les cassures ont été réalisées, dans ce cas, au niveau proximal de la constriction nucléolaire, isolant ainsi l'organisateur.

Classe D. — Le satellite est isolé avec un corpuscule Feulgen positif très rapproché de lui, non séparé par la dimension de la constriction. D'autre part, dans un grand nombre des cas, on identifie la zone de l'organisateur nucléolaire restée en connection avec le fragment centrique. Il est malaisé de définir, dans ce cas, le niveau exact des cassures

mais il est possible qu'elles puissent séparer la zone de l'organisateur nucléolaire en deux, voire plusieurs parties.

On peut résumer les fréquences des figures dans chaque classe, de la manière suivante.

En 1: de 35 à 125 h. inclusivement. En 2: de 200 à 350 h. inclusivement.

	1.		2.	
Classe A.	38.8 %	69.3=a	72.5 %	88.6=a′
Classe B.	30.5 %		16.1 %	
Classe C.	16.6 %	30.7=b	8.8 %	11.6=b′
Classe D.	14.1 %		2.8 %	

Dans les deux cas, les fréquences de cassures séparant le satellite sans l'organisateur sont supérieures aux fréquences de cassures séparant le satellite avec l'organisateur.

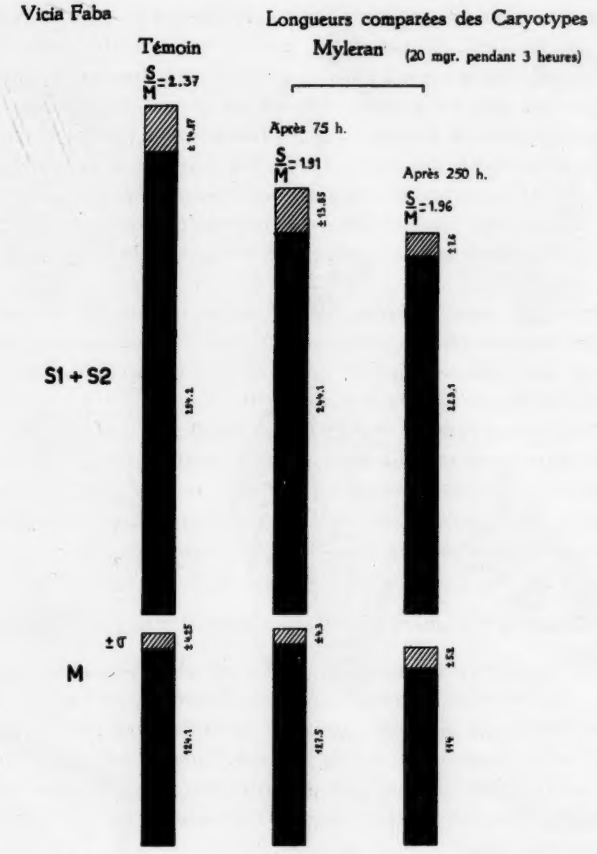
$$\frac{a}{b} \text{ et } \frac{a'}{b'} \qquad P < 0.001$$

L'analyse des tableaux précédents semble montrer que les fragments acentriques sont à peu près les seuls à pouvoir se réunir (SRC_0). Si cette observation est vraie pour toute la période où se produisent des cassures, il s'ensuit que les longueurs moyennes des caryotypes doivent diminuer, pour autant que les caryotypes déficients puissent être viables. Il est donc d'un grand intérêt de mesurer les caryotypes à divers moments du développement. Nous avons, jusqu'à présent, choisi les deux temps de 75 heures et de 250 heures, le premier parce qu'il correspond au maximum de fréquence des cassures et le second parce qu'il est situé avec certitude, dans la seconde période du cycle de mitoses où la répartition des cassures a été effectuée.

Le graphique V, résumant les résultats des mesures des caryotypes, montre que:

1) les longueurs des génomes diminuent au cours du développement;
2) les diminutions de longueurs portent surtout sur les longueurs des chromosomes S et non sur les longueurs des chromosomes M.

Il s'ensuit donc, qu'aux erreurs près, les rapports des longueurs S : M ont tendance à diminuer. Le rapport est de 2.37 chez les témoins (pour OEHLKERS, 1953, il est de 2.5). Nous pensons donc pouvoir tirer quelques indications sur les possibilités d'obtenir des caryotypes viables quoique fortement déficients.



Graphique V.

Il est intéressant de comparer les modifications de longueurs des chromosomes S et M avec les fréquences de cassures dans ces chromosomes, c'est-à-dire les graphiques III, IV et V. D'une part, le taux de cassures localisées dans le chromosome satellitique diminue, c'est-à-dire que le rapport $\frac{\text{n. de cassures dans S}}{\text{n. de cassures dans M}}$ se rapproche fortement du rapport des longueurs des chromosomes témoins, soit 2.37 ($0.30 < P < 0.40$). La distribution des cassures devient donc, en quelque sorte, proportionnelle à la longueur des chromosomes, comme pour les rayons X (à cette

différence près, que les cassures restent localisées et ne sont nullement réparties au hasard). D'autre part, la longueur relative des chromosomes M étant pratiquement invariable au cours des cycles mitotiques, on peut prévoir que les grosses déficiences de ce chromosome ont été éliminées et que les caryotypes qui les possédaient étaient inviables.

L'observation révèle que après 250 et 350 heures, on ne note pratiquement pas de chromosome M très déficient. Par contre, nous avons maintes fois observé des chromosomes S fortement tronqués, quelquefois même réduits à deux bras minuscules, c'est-à-dire de la dimension normale du bras distal.

De même que chez *Hordeum*, nous avons obtenu un certain pourcentage de cassures chromatidiques. Le pourcentage de cassures chromatidiques reste constant quelle que soit la dose utilisée et pendant la période de 250 heures après le traitement.

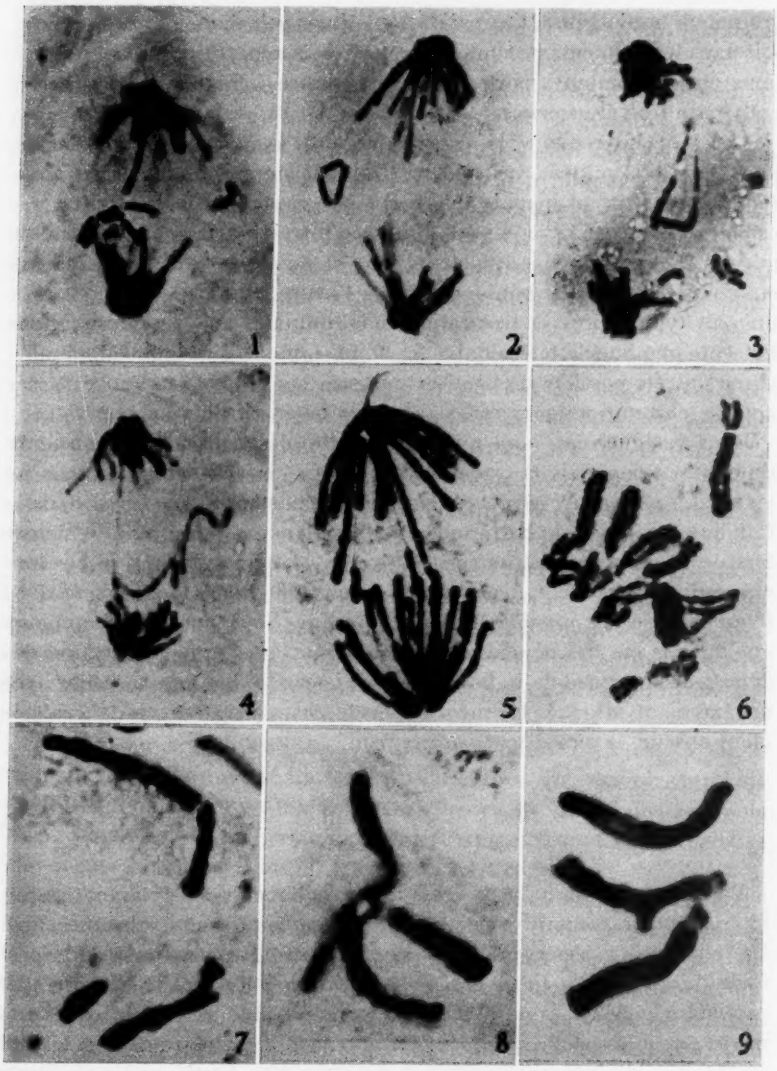
Malgré des observations répétées, nos tentatives pour mettre en évidence des translocations chromosomiques conduisant ou non à la formation de chromosomes dicentriques sont restées sans succès. Nous devons cependant mentionner l'existence d'aberrations chromosomiques qui n'ont pas encore pu être classées jusqu'à présent.

F. Examen des aberrations chromosomiques non classées

Des interchangements chromatidiques ont été observés quoique fort rarement. Au cours de la seconde mitose consécutive à l'action du toxique, nous observons des configurations triradiales et tétraradiales: environ 0,1 % de chaque type en métaphase. Comme les observations des cassures peuvent le laisser prévoir, les interchangements chromatidiques ont, dans tous les cas relevés (une 20e environ), une relation avec la localisation des cassures.

Dans la fig. 8, pl. I, les centromères des chromosomes M sont accolés l'un à l'autre. Les zones juxta-centromériques sont aussi accolées. Dans tous les cas relevés, les interchangements chromatidiques ont lieu en

Fig. 1-9. — 1. Anaphase avec débris du chromosome M; cassures isochromatidiques (1.500 ×). — 2. Anaphase avec anneau acentrique isolé entre les deux pôles (1.500 ×). — 3. Anaphase avec fragments provenant de cassures isochromatidiques (en-dessous, à droite) et chromosome M isolé entre les deux pôles (1.500 ×). — 4. Anaphase avec »pseudo-pont» et grands fragments attachés entre les deux pôles (1.500 ×). — 5. Anaphase présentant une »stickiness» et une érosion discrète des chromosomes. Les constrictions nucléolaires sont plus allongées que d'habitude. Au pôle inférieur, deux petits corps chromatiques juxta-satellitiques sont visibles (1.700 ×). — 6. Fragment acentrique d'un chromosome M en métaphase, consécutif à une cassure au niveau du segment 2 (au-dessus). — Même figure en bas, mais



dégénérative (1.700 ×). — 7. Pertubations chromosomiques diverses en métaphase X_2: satellite isolé (en bas, à gauche) et chromosome M privé de son satellite; en bas, à droite, cassure chromatidique dans un chromosome S (les fragments sont déplacés) (3.500 ×). — 8. Interchangements chromatidiques en des lieux homologues de deux chromosomes M (3.500 ×). — 9. Boucle chromatidique visible dans un chromosome S (au centre) (3.500 ×).

des points homologues. Ces points sont situés soit dans les centromères, soit dans les segments proches. Un relevé de la répartition de ces aberrations chromosomiques portant sur un nombre si réduit d'observations nous paraîtrait illusoire.

A côté des interchangements typiques, nous avons observé des aberrations chromosomiques spéciales. Elles consistent en boucles chromosomiques et doivent incontestablement être rapprochées des figures décrites par REVELL (1954) sous le nom d'intra-changements chromosomiques. Leur fréquence est basse (0,1—0,2 % des métaphases). Nous en avons observé une bonne dizaine à l'heure actuelle se rapportant à un seul type. Après la fissuration longitudinale, une des chromatides présente une boucle latérale. La fig. 9, pl. I, montre ce type d'anomalie. Il n'a pas été possible de préciser une relation entre la présence de ces boucles chromosomiques et les segments hétérochromatiques.

Dans quelques cas, nous avons pu décrire des croisements anormaux de chromatides mais la possibilité de confusion étant grande, nous ne les relaterons pas ici en détail. Un point important réside aussi dans le fait que les types d'aberrations relatées dans ce paragraphe existent lors de la première mitose consécutive au traitement. Dans ce cas, ces aberrations coexistent avec les effets physiologiques du toxique avec lesquels il serait possible de les confondre. Nous pensons néanmoins que dans le cas des boucles chromosomiques, la simple observation des détails de structure de la boucle enlève la possibilité de confusion avec un fragment attaché. Le mécanisme de ces aberrations reste évidemment obscur. Il sera discuté brièvement.

IV. DISCUSSION

Parmi les nombreux problèmes que pose l'action du Myleran, il serait intéressant de connaître tout d'abord le moment où la substance agit. Le fait que l'on observe des lésions caractéristiques au cours de la première mitose milite en faveur d'une action localisée au stade quiescent précédant celle-ci, sans qu'il soit encore possible de savoir si la première partie du stade quiescent est en cause. Cependant, toutes les observations cytologiques tendent à montrer que l'action peut persister pendant un temps plus ou moins long. Rappelons, en effet, que le maximum de cassures est observé lors de la seconde mitose consécutive au traitement et reste élevé très longtemps, du moins chez *Vicia*. On ne peut s'empêcher de penser à une accumulation de la substance dans la

graine, particulièrement dans l'écorce lipidique et à une diffusion très lente au cours du développement.

La présence de lipides en plus grande quantité expliquerait donc la plus forte sensibilité de *Vicia* au toxique ainsi que le maintien plus long des lésions. Le mécanisme de pénétration du Myleran n'est pas lui aussi sans poser de nombreux problèmes. Comment expliquer que des concentrations variant du simple au quadruple donnent la même fréquence de lésions aux erreurs expérimentales près? La faible solubilité de la substance se comportant comme une pseudo-solution pourrait rendre compte de ce fait. Nous utilisons, en fait, dans tous les cas, une solution à peu près saturée.

Une autre question que l'action du Myleran a soulevée est celle de la spécificité d'action de la substance, c'est-à-dire d'une localisation élective des cassures dans l'hétérochromatine. On sait que d'autres toxiques sont susceptibles d'avoir une même localisation. Mc Leish (1953) l'a nettement démontré pour l'hydrazide maléique. Des faits péremptoires existent aussi pour la tri-éthylène-imino-triazine et les composés di-epoxydes. Une localisation toute différente a été rencontrée par Kihlman et Levan (1951) pour la 8-ethoxycaféine. Nous avons précisé (J. et M. Moutschen-Dahmen, 1958) la localisation des lésions et leur évolution dans le cas de cette substance. Si l'on fait le bilan de toutes les substances qui, actuellement, sont susceptibles d'avoir une action localisée, on peut arriver à un nombre déjà élevé mais peu pourtant pourraient se révéler d'un usage commode en génétique.

Dans un autre ordre d'idées, l'absence de ponts vrais est certainement un des faits qui mérite le plus d'attention. Or, des observations répétées ont montré que malgré cette carence de ponts, des réunions étaient possibles. Il s'agit cependant toujours de réunions entre chromatides-soeurs survenant dans les fragments acentriques.

Jusqu'à présent, les réunions entre chromatides-soeurs dans les fragments centriques ne sont pas apparues. Quelle peut être la signification de ce fait? Les commentaires précédents ne nous permettent pas de douter qu'après l'action du toxique, l'hétérochromatine reste pendant longtemps empoisonnée. Si l'on admet que les cassures se produisent de telle manière que la totalité d'un segment hétérochromatique reste attachée au fragment centrique et que le fragment acentrique n'en contienne pas, on peut comprendre que la réunion entre chromatides-soeurs ne puisse s'effectuer qu'entre les bouts de fragments acentriques de constitution euchromatique.

Le fait de prétendre que des centromères probablement atteints par

la substance puissent interférer dans la réunion des bouts du fragment centrique nous paraît être à l'heure actuelle hautement spéculatif et ne reposant sur aucune évidence expérimentale. Il n'est maintenant pas possible de dire si l'aptitude à la réunion pourra réapparaître dans les fragments centriques au cours du développement. Nous avons souvent pensé que l'étude de toutes les phases du développement de la plante était nécessaire pour apprécier les modifications des lésions et pour le Myleran, cette étude est actuellement en cours.

Quoi qu'il en soit, l'absence de réunions des bouts centriques pendant toute la période où les cassures sont produites préside certainement aux destinées du caryotype. Cette vision a été objectivée par l'examen de cellules contenant des génomes déficients, sans que nous puissions formuler quoi que ce soit au sujet de la viabilité de ces génomes. De telles données d'ordre cytologique demandent des confirmations d'ordre génétique. Une étude génétique approfondie de l'action de Myleran est actuellement en cours de réalisation en Suède (EHRENBERG, GUSTAFSSON et collaborateurs).

Nous pouvons aussi établir un parallèle entre certains effets des rayons ultra-violets et ceux du Myleran. Rappelons, en effet, que SINGLETON (1939) a montré que, chez le maïs, les rayons ultra-violets produisent des déficiences chromosomiques surtout terminales sans produire de translocations. Chez *Gasteria*, par contre, des expériences de STRAUB (1941) ont montré la présence de translocations induites par les rayons ultra-violets et une forte localisation des cassures produisant des déficiences en des lieux proches des centromères. Ces résultats, ainsi que bien d'autres, ne sont pas sans présenter une forte analogie avec ceux obtenus par le Myleran particulièrement chez l'orge.

Dans la discussion des quelques problèmes que pose l'action de ce toxique, il nous reste à envisager l'ensemble des anomalies chromosomiques dites de type chromatidique. DARLINGTON et KOLLER (1947) ont admis que les cassures chromatidiques (B′) peuvent survenir après la fissuration des chromosomes. Nous avons employé les désignations B″ et B′, respectivement pour désigner les cassures iso-chromatidiques et les cassures chromatidiques mais sans rien présumer de la genèse de ces cassures. En 1952, THODAY a repris l'étude de cette importante question chez *Vicia faba* et a montré que les cassures iso-chromatidiques correspondent bien à un phénomène réel.

REVELL (l. c.) considère la majorité des aberrations chromatidiques comme n'étant pas explicables par la théorie classique qui place la cassure avant la réunion. Reprenant l'hypothèse des contacts émise anté-

rieurement par SEREBROWSKY (1929), il a magistralement développé des arguments en faveur de cette nouvelle explication. Dans le cas du Myleran, de l'observation précoce des intra-changements et des inter-changements chromosomiques à la déduction de l'antériorité de ces lésions, il n'y a qu'un pas. L'architecture nucléaire rapproche en effet les zones hétérochromatiques du génome et ne fait donc qu'étayer cette hypothèse. Mais en raison de l'obscurité qui pèse encore sur les questions se rapportant à l'hétérochromatine ainsi que sur le mécanisme d'action des dérivés mesyloxy-, nous ne croyons pas qu'une investigation d'ordre purement cytologique puisse être poussée davantage.

V. CONCLUSIONS

Dans le but de suivre les lésions causées par le Myleran pendant les toutes premières phases du développement radiculaire de *Hordeum sativum* et de *Vicia faba*, nous avons utilisé cette substance à des concentrations de 20 mgr., 40 mgr. et 80 mgr. pour 100 cc. d'eau pendant des temps de 45 minutes à 24 heures.

Les observations ont révélé que:

1) les trois concentrations donnent le même taux de lésions aux erreurs près. Elles correspondent certainement à des solutions saturées du toxique.
2) chez *Hordeum*, le taux de lésions chromosomiques augmente avec le temps d'action du toxique. Chez *Vicia*, il n'y a pas de proportionnalité pendant les premières mitoses. Ultérieurement, une légère proportionnalité apparaît.
3) les graines de *Vicia faba* sont plus sensibles à l'action du toxique que les graines de *Hordeum*. Chez *Vicia*, un temps d'action de 12 heures ne permet pratiquement pas le développement de la graine.
4) Pour les deux plantes, le maximum de cassures est observé à la seconde mitose consécutive à l'action du toxique. Ensuite, le taux de cassures diminue mais cette diminution est plus rapide chez *Hordeum* que chez *Vicia*.
5) Les localisations des cassures dues au Myleran ne sont pas réparties au hasard. Chez *Hordeum*, un pourcentage très élevé de cassures est observé en des endroits très voisins des constrictions. Chez *Vicia*, la majorité des cassures est localisée dans les segments chromosomiques connus comme étant hétérochromatiques.
6) Parmi les réunions chromosomiques observées chez *Vicia faba*, les seules qui ont été observées ont lieu dans les fragments acentriques

et non dans les fragments centriques. Il y a donc absence de ponts vrais. De même, chez *Hordeum*, aucun pont n'existe.

7) Nous avons noté, chez *Vicia*, la présence, quoique rare, de lésions chromosomiques du type chromatidique: cassures chromatidiques, intra- et inter-changements chromatidiques.

8) Chez *Vicia*, les caryotypes devenant de plus en plus déficients, il y a raccourcissement de la longueur totale du caryotype au cours du développement. Il est donc possible que l'on obtienne ultérieurement des plantes possédant des caryotypes fort déficients dont la structure est modelée par la répartition de l'hétérochromatine.

Nous exprimons nos plus vifs remerciements à Monsieur le Professeur Å. GUSTAFSSON qui n'a cessé, avec sa bienveillante sympathie, de nous prodiguer ses conseils fructueux et ses témoignages d'encouragement.

Nous remercions aussi la Fondation Universitaire de Belgique et l'Institut Interuniversitaire des Sciences Nucléaires sans l'appui desquels nos recherches à l'étranger n'auraient pu avoir lieu.

SUMMARY

We have followed the chromosomal aberrations induced by Myleran (di-methane-sulphonyloxy-butane) during the early phases of root tip development of *Hordeum sativum* and *Vicia faba.*

In *Hordeum*, there is a proportionality between frequency of chromosomes breaks and the duration of treatment but this proportionality is not so evident in *Vicia.* The maximum number of breaks occurred at the second mitosis following treatment and the amount then decreased. The breaks induced by the substance were strictly localized. In *Hordeum*, the maximum frequency was found near the constrictions. In *Vicia*, it was possible to conclude that the breaks were localized in the heterochromatic regions in a large majority of cases. Although Myleran is very efficient in breaking chromosomes, no true chromosome bridges were observed during the entire period of investigation. The single possible type of reunions consisted of certain acentric fragments. As a consequence of our observations, we conclude that there is a general tendency to shorten the total chromosome length in *Vicia.* Lesions of the chromatid type were very rarely detected in *Vicia.*

If the main chromosomic modifications caused by Myleran do persist and survive to the next generation, it is clear that this substance will constitute an efficient tool in cytogenetical work.

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BRIEF REPORT

A. W. F. EDWARDS[1] and M. FRACCARO: The sex distribution in the offspring of 5,477 Swedish ministers of religion, 1585—1920.

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The sex distribution in sibships, families, and populations is relevant to a number of genetical problems. The theory of the chromosomal determination of sex implies that sex is determined by chance, but there are reasons to believe that various factors disturb the random distribution of sex in sibships. It is necessary to know to what extent these factors may be neglected in practical problems.

Analyses of the data of GEISSLER (1889), have shown that there is heterogeneity between sibships in the probability of a birth being male. GEISSLER's data are well known, being quoted in most textbooks of human genetics, and having been used by FISHER (1925) as an example of fitting the binomial distribution. The analyses of GINI (1905, 1908, quoted 1951), WAALER (1928), and HARRIS and GUNSTAD (1930), support the theory of heterogeneity between sibships. Recently, however, LANCASTER (1950), reanalyzing GEISSLER's data, concluded that the heterogeneity is due to bias incurred in the collection of the data, whilst EDWARDS (1958) disputed this conclusion and considered that the data provide reliable evidence of heterogeneity between sibships.

SCHUTZENBERGER (1949), analyzing data which are second only to GEISSLER's in extent, also showed that the hypothesis of the chance determination of sex is untenable. Using their own data, BERNSTEIN (1952), and HEWITT, WEBB and STEWART (1955) came to the same conclusion, while no significant deviations from the expected chance distributions of sex in sibships were found by RIFE and SNYDER (1937), MYERS (1949), and TAYLOR (1954).

Origin of the data. — In view of the above disagreements, we decided to examine the sex composition of a peculiar sample of sibships, the offspring of Swedish ministers of religion for the period 1585—1920. These data are in the files of the State Institute for Human Genetics, Uppsala, and have been extracted from a series of collected biographies of the ministers which have been published in book form by different authors. A list of these books may be obtained from the institute. Each biography gives the years of birth and death of the parents, and the years of birth and death of the children. The data include neonatal deaths, but not still-births.

Analysis. — Families with one or more twin births have been excluded from the analysis. The sex-composition of sibships is available for 5,477 families. The sibship size ranges from 1 to 18; the total number of births is 26,037, of which 13,400 are male, and the proportion of males is therefore 0.514652. The

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TABLE 1. *Sibship size and sex composition. 5,477 families.*

No. of males	No. of children in sibship																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	279	184	89	40	22	2	2	1	0	0	0	0	0	0	0	0	0	0
1	283	383	298	186	103	41	15	10	1	2	0	0	0	0	0	0	0	0
2		190	301	271	204	130	67	42	13	2	1	0	0	1	0	0	0	0
3			127	206	200	188	114	60	46	14	8	1	0	2	0	0	0	0
4				69	119	129	119	78	49	26	16	3	2	0	0	0	0	0
5					22	64	73	89	63	48	18	11	5	1	2	0	0	0
6						11	19	35	46	25	18	14	9	3	2	0	0	0
7							4	8	22	16	13	9	13	5	2	2	0	0
8								1	6	9	7	12	3	3	1	0	1	0
9									0	4	1	3	3	2	0	1	0	0
10										0	0	1	0	1	1	0	0	0
11											0	0	2	2	0	0	1	1
12												0	0	0	0	0	0	0
13													0	0	0	0	0	0
14														0	0	0	0	0
15															0	0	0	0
16																0	0	0
17																	0	0
18																		0
Total	562	757	815	772	670	565	413	324	246	146	82	54	37	20	8	3	2	1

TABLE 2. *Contribution to the value of χ^2 for each sibship size.*

Size of sibship	Contribution to the value of χ^2	Number of classes used in calculating the contributions
1	0.276	2
2	0.792	3
3	3.036	4
4	5.369	5
5	2.914	6
6	6.989	7
7	3.518	6
8	10.455	7
9	5.385	7
10	8.532	7
11	2.438	6
12	6.736	6
13	6.015	5
14	0.012	2
Total	62.467	73

sex composition for sibships of all sizes is given in Table 1. The analysis consists of fitting binomial distributions to the data, on the null hypothesis that each birth has an independent probability (0.514652) of being male, *i. e.* that the data are homogeneous between and within sibship sizes, and in time. The significance of the observed deviations is tested by means of χ^2, the contributions from each sibship size to the value of χ^2 being given in Table 2, together with the number of classes used in calculating each contribution. For the larger sibship sizes the classes are fewer because of the pooling of those classes with expectations of less than 5; and for sibships of more than 14 the numbers are too small to contribute to the test of significance. In calculating χ^2, the total number of classes was 73, but there are 14 linear constraints due to making the expected sibship totals equal the observed, and one due to estimating the proportion of males from the data. Thus 15 degrees of freedom are lost, leaving 58. The value of χ^2 is 62.467, and there is thus no reason to reject the null hypothesis.

TABLE 3. *Variation in family size in the three different periods.*

Period	No. of families	No. of children	Mean sibship size	No. of males
1585—	1109	5774	5.206	3008
1800—	3115	14980	4.809	7642
1900—1920	1253	5283	4.216	2750
Total	5477	26037	4.754	13400

Our data are homogeneous both socially and genetically by virtue of the single occupation of the propositi, and the family tradition of ordination. The fact that the binomial distributions fit the data well implies that the data are homogeneous in time as regards sex composition, although sibship size decreases with time as is shown in Table 3. Testing the observed numbers of children in each period against the numbers expected using the mean value of 4.754 children per family, χ^2 is 126.1 on 2 d. o. f., a very significant value.

It should be noted that the good fit of the binomial distributions does not imply that the determination of sex is a chance effect, since there may be systematic variations which do not affect the distributions, and which are only detectable in a more extensive analysis of the data.

Summary. — No significant heterogeneity has been found in the sex distribution of 26,037 children from 5,477 families of Swedish ministers of religion in the period 1585—1920. The data were analyzed by fitting binomial distributions to each sibship size, using the observed proportion of males in the sample (0.514652).

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